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DIAGNOSIS OF PORTAL VEIN OBSTRUCTION; STUDIES OF INTESTINAL ABSORPTION OF GLUCOSE USING ABDOMINAL COLLATERAL VEINS*

F. TREMAINE BILLINGS JR. AND HAROLD E. DEPREE†

From the Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee and the Medical Service of Thayer Veterans Administration Hospital, Nashville, Tennessee

Received for publication May 5, 1949

Patients with ascites frequently present problems in diagnosis. It is often difficult to determine whether the ascites is a manifestation of tuberculosis of the peritoneum, carcinomatosis of the peritoneum, partial or complete obstruction of the portal vein or other conditions accompanied by the presence of fluid in the peritoneal cavity. Evidence obtained from history, physical examination and laboratory investigations may not yield sufficient information upon which a definite diagnosis may be based.

The presence of collateral venous circulation in the abdominal wall of patients with ascites may at times be helpful in establishing the diagnosis of obstruction of the portal vein. But it is surprising how often malnourished individuals with abdominal distension and free fluid in the peritoneal cavity display a prominently visible network of veins of varying size over the wall of the abdomen. These prominent veins or venules may add to the confusion regarding a diagnosis.

In the presence of portal venous obstruction, the collateral circulation carries a part or all of the blood ordinarily consigned to the portal vein. The collateral veins may, therefore, be expected to transport the products of intestinal absorption circuitously around the obstruction.

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† Formerly Senior Resident in Medicine, Thayer Veterans Administration Hospital, Nashville, Tennessee.

Presented before the Sixty-First Meeting of the American Clinical and Climatological Association, Hot Springs, Virginia, November 8, 1948.

It was the purpose of this investigation to determine whether the products of intestinal absorption could be demonstrated in the collateral veins of the abdominal wall in patients with portal vein obstruction, and if so whether such a procedure might not assist in the differential diagnosis of the etiology of ascites.

To this end the oral glucose tolerance test was employed. Blood was drawn at regular intervals after the ingestion of glucose, simultaneously from the antecubital vein and from a vein in the abdominal wall, and the blood sugar content measured.

Sixteen patients with ascites were studied. One additional patient without ascites was studied in whom obstruction of the inferior vena cava and of the portal vein was suspected and later proven to be present at operation. Two patients with no ascites and in whom there was no reason to believe portal vein obstruction was present were also studied for the purpose of obtaining normal curves. Each patient was given 100 grams of glucose by mouth while in the fasting state. The abdominal veins were often so small that a tuberculin syringe with a 24 to 26 gauge needle was used. A small amount of blood was withdrawn and transferred to an indented paraffin block. One-tenth of a cubic centimeter of this blood was carefully drawn into a micro-pipette for sugar content determination using the micro-Folin-Wu technique. Blood was not drawn exactly at the same moment from the abdominal vein and the antecubital vein. It was drawn each time first from the abdominal vein and thereafter as soon as possible (one to three minutes) from the antecubital vein.

MATERIAL

The nineteen patients studied are listed in Table I with diagnoses and results of the simultaneous glucose tolerance tests. Patient # 1 was a normal subject. Patient # 2 had an obstruction of the superior vena cava with resultant development of large veins over the upper abdominal wall. There was no evidence of liver disease, portal vein obstruction, or ascitic fluid. Figure 1 demonstrates graphically the normal simultaneous glucose tolerance curves obtained in the study of patient # 2.

Patient # 3. A 40 year old white male was admitted to the Thayer Veterans Administration Hospital in September 1946. He gave a history of anorexia, loss of

TABLE I

PATIENT	DIAGNOSIS	GLUCOSE TOLERANCE TESTS													
		Milligrams sugar per cent in blood from													
		Arm vein							Abdominal wall vein						
		Hours							Hours						
		Fast	½	1	1½	2	2½	3	Fast	½	1	1½	2	2½	3
1	Normal	71	117	123		80		70	61	120	118		70		67
2	Obstruction superior vena cava (no portal obstruction)	101	147	161		194	125		94	144	170		198	128	
3	Cirrhosis of liver, central congestive type; biopsy	74	117	142		122			70	160	166		153		
		July 21, '47							*July 21, '47*						
4	Cirrhosis of liver, portal obstructive type	80	96	131	135	132	132	131	80	131	200	197	195	202	202
		October 6, '47							*October 6, '47*						
		94	101	118		117		72	96	217	185		147		97
5	*Oct. 3, '47*—Biopsy, laparotomy, carcinoma of biliary tract; later (*March 8, '48*) of portal vein. (autopsy)	*October 3, '47*							*October 3, '47*						
		105	174	163		136			102	163	154		118		
		March 8, '47							*March 8, '47*						
		85	94	102		91			89	128	141		131		
6	Obstructing cirrhosis of liver, portal obstructive type	120	172	191		190		148	108	187	200		211		171
7	Chronic constrictive pericarditis with cirrhosis of liver, congestive type (autopsy)	108	150	144		130			99	169	185		170		
8	Cirrhosis of liver, portal obstructive type	91	171	169	143	143			89	150	182	169	163		
9	Cirrhosis of liver, portal obstructive type	97	144	177		160		167	97	139	187		178		177
10	Cirrhosis of liver, portal obstructive type	94	141	143		140		106	100	160	156		140		106
11	Carcinomatosis of peritoneum, primary carcinoma of ovary (exploratory laparotomy)	90	139		174		184		91	133		179		179	
12	Intestinal lipodystrophy (Whipple's disease) exploratory laparotomy	89		119		108	101		85		125		111	96	
13	1) Cirrhosis of liver, portal obstructive type 2) Tuberculous peritonitis (autopsy)	77	122	133		103	104		91	117	147		108	108	

TABLE 1—*Continued*

PATIENT	DIAGNOSIS	GLUCOSE TOLERANCE TESTS												
		Milligrams sugar per cent in blood from												
		Arm vein							Abdominal wall vein					
		Hours							Hours					
		Fast	½	1	1½	2	2½	3	Fast	½	1	1½	2	2½	3
14	Tuberculous peritonitis (exploratory laparotomy)	91	133		161		126		84	118		158		138	
15	Carcinoma of ovary with peritoneal metastases (exploratory laparotomy)	91	170	206	217	222	206		93	172	187	200	198	200	
16	Rheumatic heart disease, chronic general failure of circulation	98	127		156	125		101	90	114		149	132		123
17	Chronic congestive heart failure	85	120	128		132		112	96	119	148		133		115
18	Cirrhosis of liver, portal obstructive type	93	174	182	167	145			102	143	182	174	163		
19	Carcinoma obstructing inferior vena cava and extrahepatic portal vein	88	117	149	130	114	92	70	82	138	166	150	126	102	79

weight, mild watery diarrhea and indigestion accompanied by gradually increasing abdominal distension over a two months' period. He denied excessive consumption of alcohol. Physical examination revealed wasting, slight jaundice, ascites, palpable liver, and prominent superficial abdominal veins. There was no evidence of cardiovascular disease. Laboratory investigations revealed diminution of liver function with decreased total serum protein and an icterus index of 18. An exploratory laparotomy was performed and a specimen of the liver obtained. On microscopic examination there was irregular fibrosis of the parenchyma associated with intense engorgement of the veins. A diagnosis of central or congestive type of cirrhosis was made. It was believed that obstruction of the hepatic vein, cause undetermined, was present, with resultant increased pressure in the portal vein. In July 1947 the glucose tolerance tests were performed. Sugar values were consistently higher in the specimens of blood obtained from the abdominal veins than in those from the antecubital vein, Figure 2. These data were interpreted as indicating the presence of definitely increased pressure in the portal vein.

Patient # 4. A 48 year old white male was admitted to the Thayer Veterans Administration Hospital in July 1947 with a history of anorexia for six weeks, vomiting, and the onset of abdominal distenstion one week prior to admission. He had consumed large amounts of alcohol for two years prior to admission and had

had one episode of delirium tremens. On physical examination there was abdominal distension, ascites, enlarged liver and slight edema of the ankles. He was jaundiced. Laboratory investigations revealed evidence of liver damage. Total serum protein was 6.4 grams per cent with 3.0 grams of albumin. Figure 3 demonstrates graphically simultaneous glucose tolerance curves: the first, July 21, 1947; the

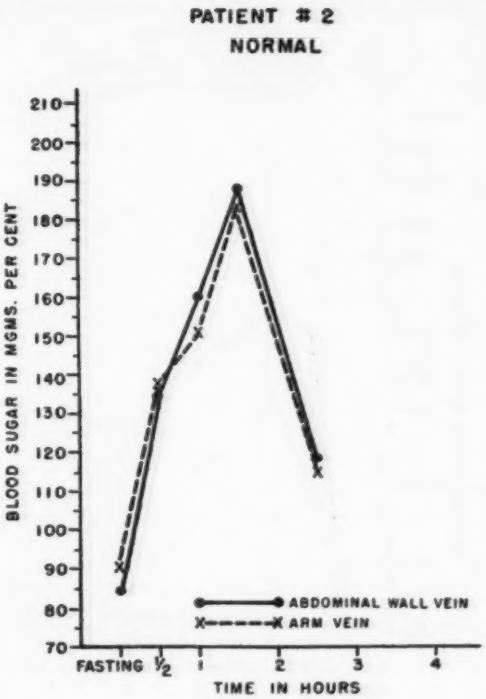

FIG. 1

second, October 6, 1947. These curves present strong evidence in support of a diagnosis of portal cirrhosis of the liver.

Patient # 5. A 25 year old white truck driver was admitted to Thayer Veterans Administration Hospital July 17, 1947 with a history of indigestion and pain below the right costal margin of six weeks duration, jaundice for five weeks, and vomiting for two weeks. The patient was markedly jaundiced. The liver was large, smooth and tender. Laboratory investigations revealed evidence of obstructive jaundice. Shortly after admission ascites was noted. On September 29, 1947 a biopsy of the

liver provided evidence of biliary cirrhosis. In October 1947 an exploratory laparotomy was performed and carcinoma of the biliary tract was demonstrated. In November 1947 an unsuccessful attempt was made to relieve the biliary obstruction. At this time the entire hilus of the liver was found to be involved in a carcinomatous mass. Autopsy examination in the latter part of March 1948 revealed complete occlusion of the portal vein by widespread adenocarcinomatosis. Figure 4

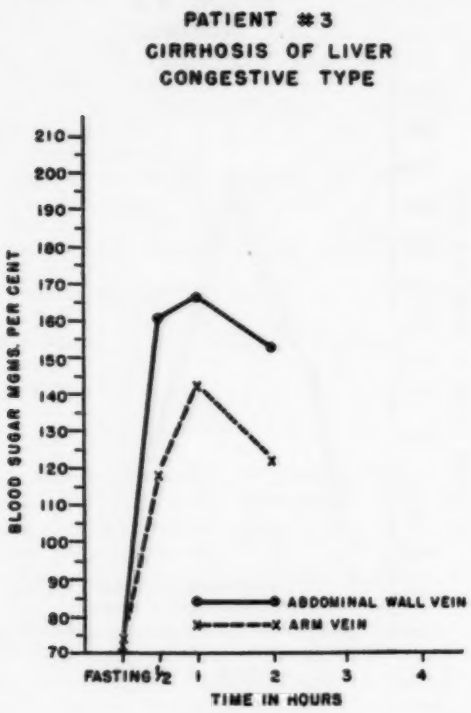

FIG. 2

demonstrates graphically two pairs of simultaneous glucose tolerance curves, one made before and the other after the development of portal obstruction. At the time of the laparotomy on October 3, 1947, there was no demonstrable evidence of portal obstruction. During the five months' period between the two tests, there had been no change in the clinical picture to indicate that portal obstruction had developed. This information, therefore, was obtained prior to the patient's death only by the use of the simultaneous glucose tolerance tests.

Patient # 6. A 53 year old white coal miner was admitted to Thayer Veterans Administration Hospital December 7, 1947 with a history of intermittent pain in the right upper quadrant of the abdomen for six years and abdominal distension for two months. He had consumed large amounts of alcohol for many years and had always eaten poorly. His abdomen was distended, there were a few distended superficial abdominal veins and the area of liver dullness was increased. There was ascites. Laboratory investigations revealed evidence of liver damage. Total serum

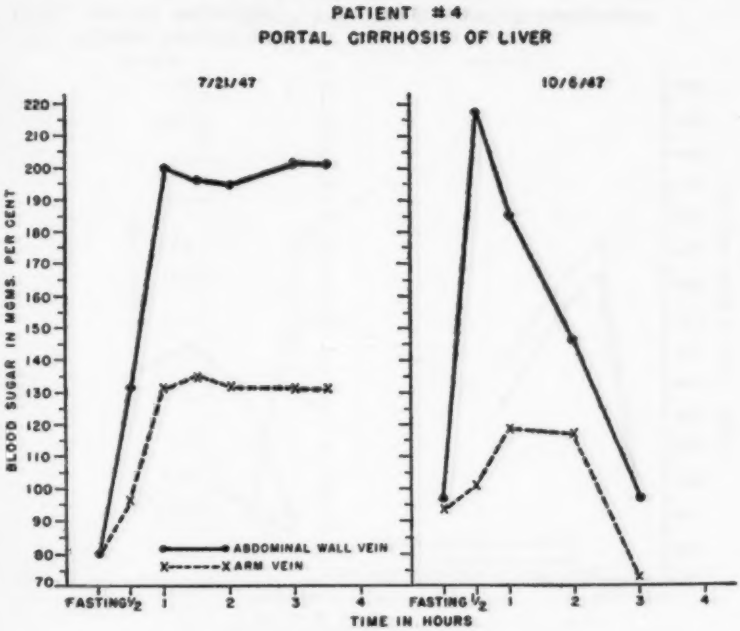

FIG. 3

protein was 5.6 grams with 2.9 grams of albumin. Simultaneous glucose tolerance tests gave blood sugar values shown in Table I which are interpreted as indicative of portal vein obstruction.

Patient # 7. A 28 year old colored laborer was admitted to Nashville City Hospital with a history of progressive abdominal distension, shortness of breath and swelling of the ankles. At autopsy he was found to have constrictive pericarditis which chiefly involved the entrance of the inferior vena cava into the right auricle. There were large superficial abdominal veins. The liver was large and there was marked congestive fibrosis. The simultaneous glucose tolerance tests revealed

evidence of increased pressure in the portal veins, Figure 5. This observation in a patient with constrictive pericarditis with, in effect, partial obstruction of the inferior cava above the liver, is of special interest because of the fact that the simultaneous glucose tolerance curves of two patients, # 16 and 17, with ascites associated with chronic congestive heart failure did not give evidence of increased pressure in the portal vein above that of the systemic veins. Moreover, this is in

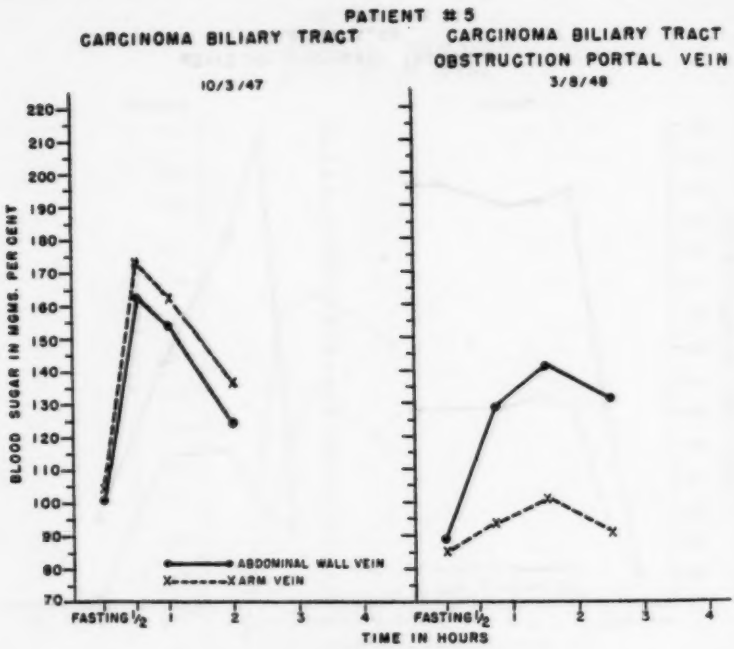

FIG. 4

contrast to the normal values obtained in the study of Patient # 2 whose superior vena cava alone was obstructed.

Patient # 8. This 52 year old white carpenter gave a history of recent indigestion and abdominal distension in association with chronic long-standing alcoholism. Physical examination and laboratory investigations indicated the presence of cirrhosis of the liver. The simultaneous glucose tolerance tests gave corroborative evidence of some portal vein obstruction, Table I.

Patient # 9. A 57 year old grocery clerk was admitted to Thayer Veterans Administration Hospital March 2, 1948 complaining of indigestion and abdominal

distention. He had consumed large amounts of alcohol and had a poor appetite for many years. There was ascites, some distension of superficial abdominal veins and a palpable liver which seemed to be nodular. Laboratory investigations revealed evidence of liver damage. Total serum protein was 6.1 grams per cent with 2.9 grams of albumin. The simultaneous glucose tolerance curves in Figure 6 gave suggestive but not conclusive evidence of some portal vein obstruction.

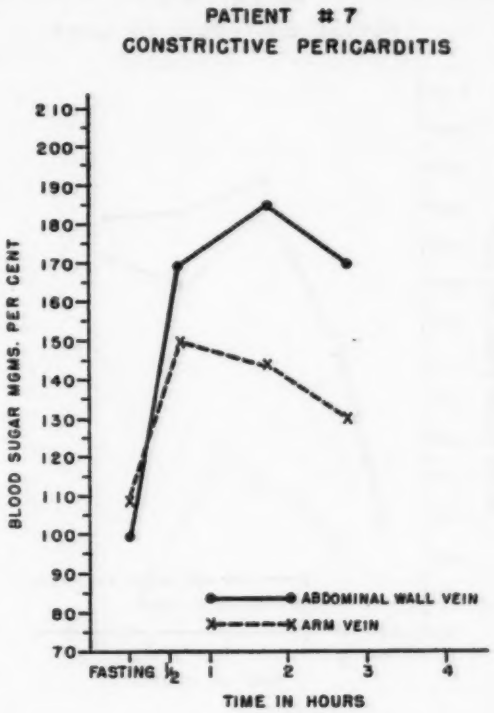

FIG. 5

Patient # 10. A 36 year old white housewife was admitted to Vanderbilt University Hospital February 2, 1948 complaining of intermittent abdominal distension and indigestion for seven months. She denied excessive consumption of alcohol. She had been treated for syphilis thirteen years ago over an eighteen month period. Physical examination revealed evidence of weight loss, several spider angiomata, abominal distension, ascites, and although the liver was not palpable the area of liver dullness was enlarged to percussion. The Wassermann

test was positive. Laboratory investigations revealed evidence of diminished liver function. Total serum protein was 5.9 grams per cent with 2.02 grams of albumin. Simultaneous glucose tolerance test gave blood sugar values, shown in Table I, which were not conclusive. Although this patient almost certainly had Laennec's cirrhosis of the liver, portal hypertension may not have been present. If collateral

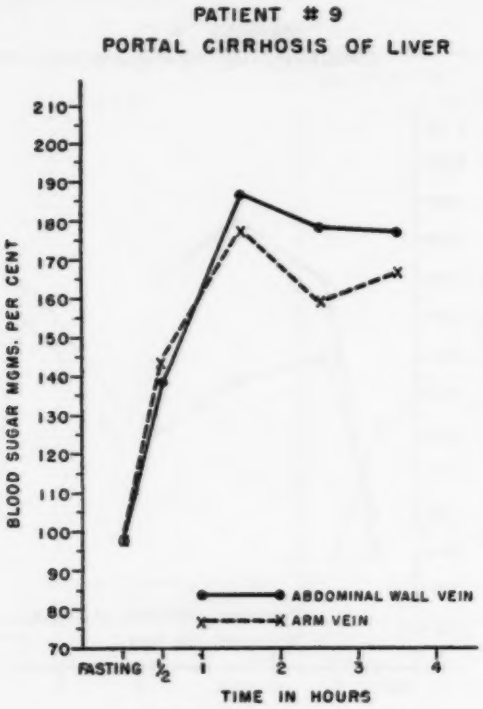

FIG. 6

circulation was present it was not demonstrable in the veins of the anterior abdominal wall.

Patient # 11. A 43 year old housewife was admitted to Vanderbilt University Hospital complaining of progressive abdominal distension over a four months' period. Physical examination revealed ascites and deviation of the cervix to the left. There was a hard nodule palpable in the cul de sac on rectal examination. Exploratory laparotomy was performed and widespread adenocarcinomatosis originating in the right ovary with metastases throughout the peritoneal cavity

was demonstrated. Simultaneous glucose tolerance tests carried out prior to operation yielded data which indicated that there was no portal vein obstruction, Table 1. This was confirmed at operation.

Patient # 12. A 47 year old farmer was admitted to Vanderbilt University Hospital on October 18, 1947 complaining of upper abdominal pain, shortness of breath, diarrhea and abdominal swelling. He had had indigestion and abdominal

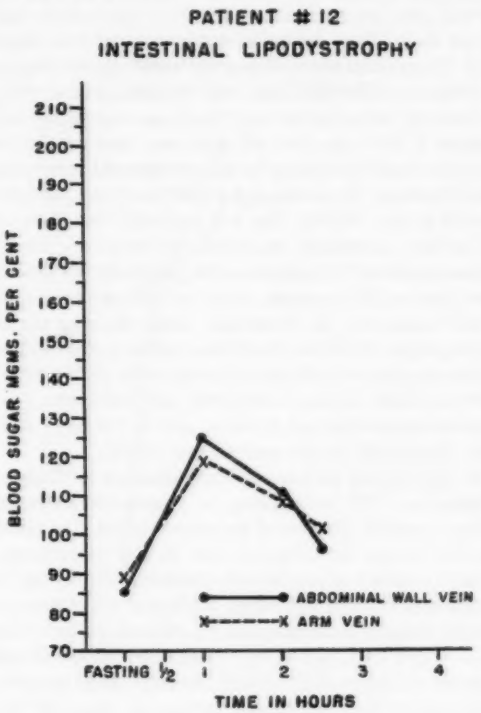

FIG. 7

cramps for four and a half years and for two months had noted gradual increase in the size of his abdomen. He had abstained from alcohol for eight years, although prior to that time he had been a moderately heavy drinker. He had lost forty pounds during the present illness. On physical examination there was evidence of weight loss, cachexia, scattered petechiae over the skin of the legs, abdominal distension with ascites, enlarged liver and spleen. There was no evidence of collateral circulation. Laboratory investigations revealed very little evidence of liver disease.

Total serum protein was 4.63 grams per cent with 2.6 grams of albumin. In spite of the lack of confirmatory laboratory data, it was the opinion of those who examined him that this patient had cirrhosis of the liver with portal vein obstruction. Simultaneous glucose tolerance tests failed to produce evidence of portal vein obstruction, Figure 7. Following paracentesis a mass thought to be retroperitoneal was felt to the left of the midline in the upper abdomen. An exploratory laparotomy was performed on November 24, 1947. The typical picture of intestinal lipodystrophy was observed and was confirmed on microscopic examination of a mesenteric lymph node. There was no evidence of portal vein obstruction.

Patient # 13. This patient was a 60 year old housewife who was closely followed at Vanderbilt University Hospital from May through October 1947. She was admitted three times for investigation and finally an exploratory laparotomy was performed October 2, 1947. She died ten days later because of a cerebrovascular accident. Her course was characterized by the development of indigestion, anorexia, and progressive abdominal distension and weight loss over a period of six months prior to admission to the hospital. She was cachectic, and there was abdominal distension and ascites. Laboratory investigations revealed evidence of liver disease. Paracenteses consistently yielded a turbid pseudochylous fluid. Serologic tests for syphilis were positive. At operation, numerous enlarged tuberculous mesenteric lymph nodes were observed. The lymphatic vessels draining the intestines were obstructed and engorged. Moreover, there was nodular portal cirrhosis of the liver. Prior to operation simultaneous glucose tolerance tests, yielded blood sugar values which are shown in Table I. The results were not conclusive. At operation two factors, tuberculous peritonitis and cirrhosis, one or the other or both of which could have been responsible for the ascites, were found.

Patient # 14. This 41 year old housewife was admitted to Vanderbilt University Hospital in September 1947 complaining of progressive abdominal distension, weakness and loss of weight. She denied the use of alcohol. The family history was noteworthy in that several close relatives had died of tuberculosis. Physical examination revealed evidence of weight loss, abdominal distension, the presence of ascites and hepatomegaly. The superficial abdominal veins were prominent. Although laboratory investigations revealed no evidence of liver dysfunction, the presence of an enlarged liver, ascites and prominent abdominal veins seemed to point to a diagnosis of Laennec's cirrhosis of the liver. Some observers were of the opinion that the patient had tuberculous peritonitis. Acid fast bacilli were not demonstrated in the ascitic fluid, the specific gravity of which was border line between that of a transudate and an exudate. Simultaneous glucose tolerance tests, Table I, showed no evidence of portal vein obstruction. Exploratory laparotomy revealed extensive tuberculous peritonitis. There was no obstruction of the portal vein.

Patient # 15. This 40 year old housewife was admitted to Vanderbilt University Hospital first in May 1947 complaining of abdominal distension, loss of weight and weakness for the preceding three years. The course of her illness was marked by its chronicity and by the remarkable disappearance and reappearance of ascites.

Although tests of liver function were for the most part within normal limits, a diagnosis of Laennec's cirrhosis was made. She had always had poor dietary habits. She denied the use of alcohol, but had consumed large amounts of turpentine orally for contraceptive purposes. Simultaneous glucose tolerance curves, Figure 8, revealed no evidence of portal vein obstruction. Finally in October 1948, following a paracentesis, asymmetry of the abdomen was noted and on pelvic examination a mass in the region of the left ovary was felt. Extensive peritoneal metastases

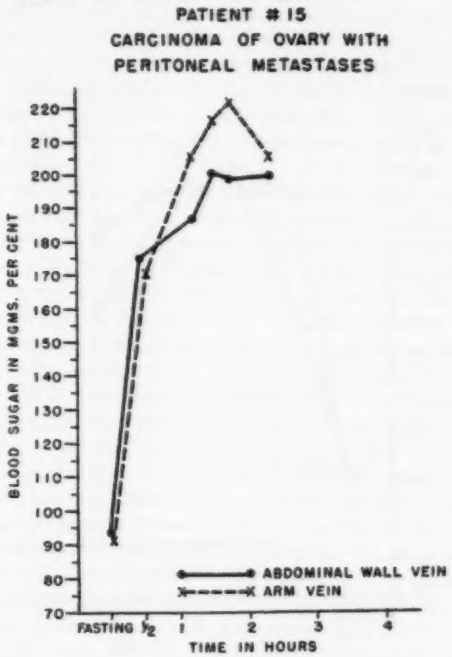

FIG. 8

from an ovarian cystadenocarcinoma were demonstrated when an exploratory laparotomy was performed in November 1948. The portal and mesenteric veins were carefully examined. They were normal.

Patient # 16. This 28 year old white male was continuously confined to bed in Vanderbilt University Hospital for almost a year. He had had acute rheumatic fever as a child with resultant mitral insufficiency and stenosis. There was marked chronic congestive heart failure, and persistent ascites, associated with massive peripheral edema. Simultaneous glucose tolerance tests revealed no evidence of

portal vein obstruction as shown in Table I. Examination at autopsy revealed a huge congested liver with the typical changes associated with chronic passive congestion due to heart failure.

Patient # 17. This 60 year old colored female had been followed for many years at Vanderbilt University Hospital. She developed congestive heart failure on an

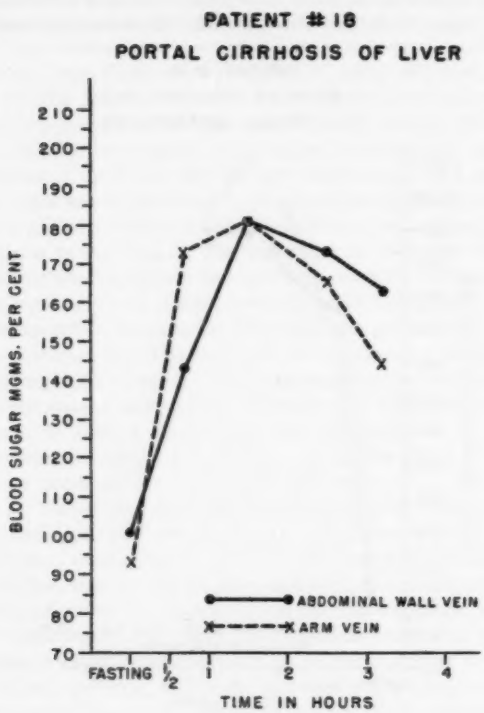

FIG. 9

arteriosclerotic basis with enlargement of the liver and ascites. Simultaneous glucose tolerance tests, Table I, revealed no evidence of portal vein obstruction.

Patient # 18. A 59 year old white male was first admitted to Vanderbilt University Hospital with tremendous abdominal distension which had gradually become more and more apparent for six months prior to admission. He had periodically consumed large amounts of beer and whiskey and had had a very poor diet for many years. He gradually became weaker, eventually lost his appetite altogether and on admission was completely exhausted. He was a rather thin man ex-

cept for his huge abdomen, the maximum circumference of which measured six feet. The abdominal wall was very tightly stretched by the large amount of fluid present in the peritoneal cavity. Laboratory investigations revealed marked impairment of liver function. Total serum protein was 6.9 grams per cent with 3.42 grams of albumin. Although there was convincing evidence that this patient had Laennec's cirrhosis of the liver with portal vein obstruction, the simultaneous glucose tolerance curves were not significantly different one from the other, Figure 9. It is probable that the marked intra-abdominal pressure and distension of the abdominal wall were such as to result in raising the pressure in the veins of the abdominal wall above that of the portal vein. Under such circumstances collateral circulation could not be maintained through the abdominal veins. Unfortunately the validity of this explanation could not be tested by repeating the examination after the removal of a quantity of ascitic fluid, thus lowering the intra-abdominal pressure and abdominal wall tension. No veins were visible in the abdominal wall after paracentesis.

Patient # 19. A 47 year old white farmer was admitted to Vanderbilt University Hospital on January 24, 1949 complaining of pain in the flanks, weakness and weight loss. He was thin, there was marked dilatation of the veins of the abdominal wall, the liver was enlarged and there was a firm fixed mass palpable deep in the abdomen above the umbilicus. There was brawny induration of the skin and subcutaneous tissues of the feet and ankles. Venous pressure in the arm was sixty millimeters of waters. In the lesser saphenous vein of the leg the pressure was one hundred thirty five millimeters of water. A tentative diagnosis of retroperitoneal malignancy with obstruction of the inferior vena cava was made. Simultaneous glucose tolerance tests were carried out. Sugar levels in the blood obtained from the vein of the abdominal wall were consistently higher than those in blood from the antecubital vein, Table I. On this basis, extrahepatic obstruction of the portal vein was suspected in addition to obstruction of the inferior vena cava. At operation, the mass was discovered to be composed of matted lymph nodes and omentum through which the portal vein and its tributaries passed. The pressure in the portal vein above the lesion was eighty millimeters of water; below the mass in the superior mesenteric vein, the pressure was two hundred eighty millimeters of water. This seemed to be confirmatory data for the clinical diagnosis of portal vein obstruction. The inferior vena cava was not visualized. Microscopic examination of the biopsy material revealed metastatic carcinoma of undetermined origin. No site of origin was discovered before the patient was discharged from the hospital.

DISCUSSION

Sherlock and Walshe (1) reported the results of studies of intestinal absorption of carbohydrate, fat and amino acids in a patient with cirrhosis of the liver in whom an enlarged abdominal wall vein communicated with the portal venous system. The patient did not have ascites but the data obtained from clinical history, physical examination,

laboratory investigations and a biopsy of the liver conclusively supported a diagnosis of Laennec's cirrhosis. These investigators were able to demonstrate higher blood sugar values in the abdominal wall than in the antecubital vein after administration of oral glucose. They concluded from this data that the vein in the abdominal wall carried blood from the mesenteric veins.

We were able to duplicate some of the results of Sherlock and Walshe. Moreover the procedure appears to offer a practical adjunct to other methods of diagnosing portal vein obstruction regardless of the etiology.

The procedure may occasionally be rather tedious and time consuming. Abdominal veins, even in the presence of portal vein obstruction, are often small and multiple samples of blood may be obtained from them only with difficulty.

Several factors may tend to make results misleading or inconclusive. Collateral circulation in the presence of portal vein obstruction may not develop through the veins of the abdominal wall. When distension or ascites are present, the pressure in the superficial abdominal veins may actually be higher than in the portl vein itself. Moreover, admixture of systemic blood in the abdominal veins may be sufficient to obscure the presence of a small increase in blood sugar levels. Nevertheless, our experience indicates that the procedure is often helpful and sometimes diagnostic.

Interpretation of the blood sugar curves does not always lead to clear conclusions. There are variations between fasting sugar levels in the blood of the same patient taken almost simultaneously from the antecubital and abdominal wall vein which can only be accounted for by the difficulties frequently encountered in obtaining blood from small abdominal veins. But if the sugar levels in the blood obtained from the abdominal wall veins are consistently higher than those in the blood from the antecubital vein throughout the test, the results may be significant. For example, if the average elevaton of sugar level in blood from the abdominal wall vein is at least fourteen milligrams per cent higher than in blood from the antecubital vein for four determinations made during the one to three hours after ingestion of glucose, the difference is statistically significant, and may be taken to indicate shunting of portal blood through the veins of the abdominal wall.

(The average elevation based on two successive readings would have to be at least twenty milligrams per cent higher and the average elevation based on three successive readings would have to be at least sixteen milligrams per cent higher to be statistically significant.) This was apparently true in seven of the eleven cases here reported in which a diagnosis of portal vein hypertension was compatible with clinical and anatomical findings (patients #3, 4, 5, 6, 7, 8, 19). In patients #9, 10, 13 and 18 in whom portal cirrhosis was present, results were inconclusive. Of the eight patients in whom portal hypertension was not compatible with clinical and anatomical findings (patients #1, 2, 11, 12, 14, 15, 16 and 17 and in addition the first studies of patient #5), there was no statistically significant elevation of sugar levels in blood from the abdominal wall veins over levels in blood from the antecubital vein.

SUMMARY

Simultaneous measurements of blood sugar levels in the antecubital and superficial veins of the abdominal wall of sixteen patients with ascites and one with portal vein and inferior vena caval obstruction without ascites, were made at intervals following the ingestion of glucose. Two additional patients were used as controls. In the presence of hypertension of the portal vein, higher levels of sugar were often demonstrated in blood from the veins of the abdominal wall than in blood from the antecubital vein. Our experience indicates that this procedure is frequently helpful in determining whether or not portal vein obstruction is present.

The authors wish to acknowledge the assistance rendered by Doctor Margaret P. Martin in statistically interpreting the blood sugar curves.

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TREATMENT OF CUTANEOUS CARCINOMA WITH PODOPHYLLIN*

Preliminary Note

MAURICE SULLIVAN, M.D.

From the Department of Medicine (Division of Dermatology) and the Department of Pharmacology of the Johns Hopkins University School of Medicine

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The purpose of this communication is to report brief observations of the manner in which cutaneous carcinomas undergo involution following the topical action of podophyllin.

Podophyllin produces arrest of mitosis, distortion of nuclear pattern and a chain of cytotoxic effects (1) (2) (3) in the epithelium of normal skin as well as in the epithelial cells of any benign or malignant skin tumors with which it comes into contact for 8 hours or longer (1) (4) (5). There is abundant clinical (6) (1) and microscopic evidence (1) (2) (9) that the drug's cytotoxic effects are more selective for certain types of epithelial cells, particularly those of moist, young, non-keratinized condylomata acuminata which are rapidly destroyed by a single application. By contrast the action on surrounding normal skin, unless excessive and unduly prolonged, is reversible (1) (2) (4) as macroscopic and microscopic integrity of the skin is established soon after removal of the resin. Selective damaging effects on mouse tumors (7) and on mouse tumor cells in tissue culture (8) result from exposure to the drug. Podophyllin and podophyllotoxin are more toxic for recently weaned mice than for adult mice (5); the toxin is extremely toxic for chick embryoes (5). Dry keratinized verrucae vulgares and chronic, or old, partially keratinized condylomata acuminata are resistant to the rapid tumor necrotizing action. The aforementioned facts indicate that the cellular destructive action is more selective for young, embryonic and tumor cells than for adult cells and that the changes produced in adult cells are reversible within certain limits.

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The cancericidal potentialities were first pointed out when the similarity of colchicine and podophyllin effects was discovered (9). As a prelude to the clinical use of podophyllin for cutaneous carcinoma, studies in this laboratory (5) have accumulated sufficient data during the past 2 years to show that the topical application of podophyllin for prolonged periods is safe, provided contact with the eyes is avoided. The response of cutaneous carcinoma is slower than that exhibited by condyloma acuminatum and much more of the drug is required, but it appears from preliminary observations to be equally as impressive and as complete. Thus far 15 patients having a total of 25 cutaneous carcinomas have been treated. Biopsies were secured from each patient prior to treatment. Follow-up of 5 lesions was not accomplished because serial sections were excised at various stages of involution for microscopic analysis of treatment effects. Of the 20 lesions which were destroyed by multiple applications and permitted to heal, all except one may be considered satisfactory results. In this group there were 12 basal cell carcinomas measuring 0.5 cm., 0.8 cm., 1.5 cm., 1.5 cm., 1 x 2 cm., 2 cm., 2 cm., 2 x 2.5 cm., 2 x 3 cm., 2 x 3 cm., 2.5 x 3 cm. and 12 x 14 cm.; 7 intra-epidermal carcinomas measuring 2 cm., 2 cm., 3 cm., 4 cm., 4 cm., 4 x 6 cm. and 8 x 14 cm.; and 1 prickle cell carcinoma, 4 x 6 cm. The 12 x 14 cm. basal cell carcinoma was the only failure in the group. The resin was applied topically as a 25 percent suspension in mineral oil or a 20 percent solution in alcohol. Numerous treatment schedules were tried; these varied from 2 applications in 7 days to 50 applications in 3 months. To date a satisfactory average schedule has not been determined but probably approximately 10 to 20 applications within 3 weeks will be adopted as a working schedule based on current trial and error results. The periods of involution of treated tumors varied considerably. The most rapid disappearance was in 10 days; the slowest was 3 months. It must be emphasized however, that varying experimental treatment schedules were being observed and that over treatment in more than half of the cases undoubtedly prolonged healing in some cases. Including such variables the average healing time was 49 days after the first application. A most impressive clinical observation is the selective ulceration of tumor tissue; the borders of treated basal cell carcinomas are chanellized by the drug and the delineation of ulcerative moats

outlining previous tumor sites is clear cut in contrast to the superficial and reversible signs of injury to the surrounding normal skin. The treated area heals with a pliant, slightly atrophic scar which resembles the cosmetically successful roentgen irradiation scar when it occurs without accompanying telangiectasia. The follow-up periods vary from one to eight months with an average of approximately six months.

The results herein reported are encouraging, but it is obviously premature to express enthusiasm for the method until sufficient time has elapsed to determine whether the results will be permanent. Toxicologic, pharmacologic and pathologic studies are being conducted (5) in conjunction with clinical studies to ascertain the tolerance of the drug, the time factors and amount of drug necessary to destroy cutaneous cancer permanently without causing irreversible damage to circumjacent normal tissue and at the same time avoiding hypersensitivity which is frequently produced by the drug (1) (10). The selective topical effects of podophyllin differ from the effects of other chemicals such as those utilized in the chemosurgical method of Mohs, which produces non-selective tissue destruction that is measured by microscopic examinations of the fixed and damaged tissues as they are removed. The anticipated objections to the use of podophyllin are two features that may be viewed by some as advantages, namely, simplicity and economy. The lack of available fixed tissue for microscopic examination during treatment may be an objection voiced by advocates of Mohs' chemosurgical microscopically controlled method. Therefore, it is not the intention of the author to recommend podophyllin as a treatment of carcinoma to supercede other well known methods such as surgery, roentgen therapy, electrodesiccation, cauterization or chemosurgery. It is hoped that other investigators may explore this method, duplicating our approach (4) to the problem, which consists of excising treated tumors in various stages of involution and after healing to determine sequential microscopic signs of the tumor necrotizing effect and the permanency of the destruction. Detailed descriptions of our findings (4) will be published next year after we have accumulated sufficient clinical and microscopic data to justify proper evaluation of the method.

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Addendum—Two other preliminary reports of the use of podophyllin in the treatment of cutaneous carcinoma and senile keratoses have been submitted recently by Dr. Leslie Smith of El Paso, Texas and Dr. Fletcher Hall of Los Angeles, California to the Archives of Dermatology and Syphilology. The results of Smith and Hall (In Press) are essentially similar to those herein reported.

M. S.

A COMPARISON OF SARCOIDOSIS AND TUBERCULOSIS WITH RESPECT TO COMPLEMENT FIXATION WITH ANTIGENS DERIVED FROM THE TUBERCLE BACILLUS

WILLIAM H. CARNES[1] AND SIDNEY RAFFEL[2]

The Departments of Pathology and of Bacteriology and Experimental Pathology, Stanford University, School of Medicine

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The etiology of sarcoidosis has not been established although an imposing number of observers have proposed arguments in favor of the theory that it is a form of tuberculosis. Several reviews in the past few years have discussed the principal evidence for and against this theory (1–8). The most significant facts to date regarding the possible relationship of these diseases are the following.

There is a striking similarity in the pathological lesions of sarcoidosis and tuberculosis. The epithelioid-cell tubercle is the characteristic histological unit of each. The lack of caseation in the lesions of sarcoidosis is the pathological feature that distinguishes it most clearly from typical tuberculosis. However, caseation does not invariably occur in the lesions of tuberculosis (9) so that its absence from the lesions of sarcoidosis is not an absolute differentiation. Moreover, it has been reported that patients with sarcoidosis react to the inoculation of heat-killed (10) or avirulent (11) tubercle bacilli with the formation of noncaseous tubercles in contrast to normal individuals in whom caseation occurs. Since caseation in tuberculous lesions is dependent in large part upon hypersensitivity of the tissues to tuberculoprotein, its failure to occur in sarcoidosis might conceivably be related to the fact that the great majority of cases of sarcoidosis are not hypersensitive to tuberculin. It has been reported, furthermore, that patients with sarcoidosis fail to develop hypersensitivity to tuberculin following vaccination with avirulent tubercle bacilli (11, 12, 13) as normal individuals do. There are a number of other clinical and pathological features of sarcoidosis that are not typical of tuberculo-

[1] Present address: The Johns Hopkins Hospital, Baltimore, Md.

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sis (1–9) but the most persuasive evidence against a tuberculous etiology is the repeated failure of the great majority of investigators to identify the tubercle bacillus in the lesions of the disease by staining, culture and inoculation of tissue into a variety of animal species (1, 9, 14). Nevertheless an impressive proportion of fatal cases of sarcoidosis have proven to have typical tuberculosis at autopsy as well as typical sarcoidal lesions (1, 2, 5, 6, 8). The coexistence of both diseases in the same patient has been invoked by some as evidence of their identity and by others as evidence of their distinct individuality. Some more specific evidence is needed before the etiological relationship of these diseases can be established.

Antibodies to *M. tuberculosis* have been identified in the serum of tuberculous patients with considerable frequency (15, 16, 17, 18). Antibodies may also be demonstrated in the serum of infected animals throughout the course of infection and often persist when demonstrable skin sensitivity to tuberculin has waned (19). If sarcoidosis were in fact due to the tubercle bacillus it might be anticipated that antibodies to the organism would be found in these patients even in the absence of tuberculin sensitivity. The present study was undertaken in order to determine whether complement-fixing antibodies to *M. tuberculosis* occur in the serum of patients with sarcoidosis with sufficient frequency to indicate an etiological relationship of this agent to the disease.

CASE MATERIAL

A series of twenty-two cases of sarcoidosis has been tested. The diagnosis was supported in each instance by biopsy of at least one involved tissue. There were no autopsied cases. In only one case, to be described below, was any bacteriological evidence of tuberculosis found during the course of this study. The distribution of the lesions, demonstrated by clinical, roentgenographic and pathological means, was similar to that found by others in larger series of cases (6, 20, 21). The positive observations are recorded in Table 1. Every patient had a roentgenogram of the chest. Enlarged hilar lymph nodes and pulmonary infiltrations were considered evidence of the presence of the lesions in these sites. The pulmonary lesions were usually of miliary type but occasionally they were patchy and asymmetrical. No cavities

TABLE 1

Pertinent clinical, radiological and pathological data on the cases of sarcoidosis

CASE NUMBER		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Age (yrs)		29	23	48	25	25	34	26	47	19	24	24	19	32	30	31	49	28	22	65	35	21	44
Sex		F	F	F	M	M	M	M	F	M	M	M	M	F	F	M	F	M	M	F	F	F	F
Race		W	W	W	N	N	W	N	W	N	N	N	N	W	N	W	N	N	N	W	N	N	W
Tuberculin test (mg. Old Tuberculin)	Positive									±			0.1							0.1	1.0	1.0	
	Negative	1.0	0.1	.01	1.0	0.1	10.	10.	10.	1.0		10.		10.	1.0	1.0		.01	.01				1.0
Guinea-pig inoculation*		Neg.				Neg.	Neg.	Neg.	Neg.	Neg.		Neg.	Neg.	Neg.	Neg.	Neg.	Neg.‖	Neg.		Neg.	Neg.	Neg.	
Distribution of lesions	Lymph nodes:																						
	Superficial§					+	+	+	+		+	+	+		+		+	+	+	+	+	+	
	Mediastinal†	+		+	+					+	+	+	+		+	+§		+	+		+	+	
	Abdominal§		+																				+
	Lungs†			+	+	+						+	+		+	+	+	+	+		+	+	+
	Eyes‡	+	+	+	+	+				+						+		+	+	+			
	Skin§	+		+	+			+						+	+								
	Parotid gland§	+			+	+				+			+										
	Bones†																				+		
	Mucosae§		+									+											
	Spleen§																						+

* Inoculation with portion of biopsy material which contained lesions characteristic of sarcoidosis.

† Diagnosed by roentgenographic examination.

‡ Diagnosed by ophthalmoscopic examination.

§ Lesions verified by pathological examination of biopsies.

or apical lesions typical of tuberculosis were found. The lesions of the eyes consisted of active or healed uveitis and one case had in addition a retrobulbar neuritis. The biopsies demonstrated numerous epithelioid cell tubercles with no more than minimal microscopic necrosis. Carbolfuchsin stains in every instance failed to reveal acid-fast bacilli. Portions of the biopsy material from 16 cases were inoculated into guinea pigs and none of the animals developed tuberculosis. With one exception no acid-fast bacilli were obtained from any source in this group of patients. The exception was a 19 year old negro youth (case 9) with typical uveoparotid fever, enlarged mediastinal lymph nodes and clear lung fields. His tuberculin test to 1 mg. of Old Tuberculin was equivocal. Smears of his sputum had been negative but one guinea pig, inoculated with material from a gastric lavage, developed tuberculosis. A portion of his parotid gland, which contained lesions typical of sarcoidosis, was inoculated into two other guinea pigs and yielded a negative result. The patient moved away before the gastric lavage could be repeated.

Records of tuberculin tests were available in all but two of the cases. The results are summarized in Table 1. None reacted to 0.01 mg. dose of Old Tuberculin. Two of the 16 who were tested with 0.1 mg. gave positive reactions. Two others who were tested with 1.0 mg. reacted. Five who were tested with 10 mg. were negative. Guinea pigs were inoculated with biopsy material from every case which had a positive tuberculin test.

The ages of the patients ranged from 19 to 65 years and the mean age was 28 years. There were equal numbers of men and women. Thirteen cases (59%) were negroes.

For comparison with the cases of sarcoidosis, a group of 26 cases of active tuberculosis was selected. This group included cases with pulmonary, renal, osseous and meningeal infection. The diagnosis in each instance had been confirmed by the demonstration of acid-fast bacilli in the sputum or other material. Only one of these cases had a negative tuberculin test. This case will be referred to again later. Further comparison was made with two groups of healthy preclinical medical students who had recently been tuberculin tested. The first group of 30 students reacted positively to the first test dose of P.P.D. Each one had recently had an x-ray of the chest and none had clinical or

roentgenographic evidence of active tuberculosis. The second group of 29 students was negative to the second test dose of P.P.D. and none had a history of tuberculosis or of a previously positive tuberculin test.

METHODS

There is a large body of data on the complement fixation reaction in patients with tuberculosis. The wide variation in the results of early investigators (18) has been greatly reduced in the more recent studies employing better standardized methods (22). In 1927 Pinner (15) summarized the results of complement fixation in some 27,000 reported tests from the earlier literature and his own experience. The variety of antigens used included culture filtrates, Old Tuberculin, whole bacillary cells, partially defatted bacilli, lipoidal fractions, various watery extracts and polysaccharide. His analysis indicated that the isolated protein of the organism was not a satisfactory antigen for complement fixation and that the alcohol soluble substances were the best. More recent experience indicates that an antigen of partially defatted bacilli gives positive results in 60–70% of cases of active tuberculosis (22). It was anticipated that the chemical methods employed in the earlier studies may have yielded fractions of somewhat different antigenic properties from those obtained by more recent methods. In the present study therefore a wide variety of antigens was used in order to provide a general coverage of the types of antigenic material used by others in the past as well as to provide certain of the more striking chemical components of the organism in high concentration. All antigens were prepared from the H_{37} strain of tubercle bacilli. Except where otherwise indicated the bacilli were cultured on a synthetic medium of the Long-Seibert type containing asparagine.

Heat-killed bacilli. Organisms cultured on synthetic medium over a period of a month were washed free of medium, ground in a mortar, and suspended in isotonic salt solution in a concentration of 10 mgs. per ml. This suspension was heated at 65°C. for one-half hour and was preserved by the addition of Zephiran (1:10,000). Dilutions of 1:50 or 1:100 of this suspension were employed in 0.10 ml. amounts in the tests.

Defatted bacilli. Bacilli were partially defatted in accordance with

the method of Kolmer (22). A four week culture in glycerol-veal broth was washed with distilled water, dried over sulphuric acid, and ground to a powder in a mortar. Each gram of bacillary powder was extracted with 200 ml. of ether followed by 200 ml. of acetone and then 200 ml. of absolute alcohol. In each case boiling was continued for one hour on an electric heater with a Liebig condenser. All the organic extracts were discarded. The dried bacillary residue was made up in a concentration of 1 gm. per 200 ml. of water. This was boiled for one hour and then made isotonic by the addition of sodium chloride. Phenol was added to a concentration of 0.25% as a preservative.

Culture medium filtrate. This antigen was the whole filtrate of a glycerol-veal broth culture prepared according to the technique of Petroff (17). The culture was filtered through several layers of paper; the filtrate was heated at 65°C. for one-half hour and was then placed in sealed vaccine bottles which were heated at 60°C. for thirty minutes.

Culture medium protein. Cultures were grown for eight weeks on synthetic medium. Protein was prepared from the culture fluid by the method of Seibert and Glenn (23) without the use of heat. The protein was precipitated by half saturation with ammonium sulphate at pH 7 and was reprecipitated eight times. The final product was lyophilized and freshly prepared solutions in M/15 phosphate buffer of pH 7.3 were used for each set of tests.

Bacillary protein. Cultures were grown on synthetic medium. The bacilli were washed, dried, ground and defatted as described above. Protein was extracted with phosphate buffer (pH 7.3) and purified by reprecipitation several times from half saturated ammonium sulphate at pH 7. The purified protein was lyophilized and freshly prepared solutions in M/15 phosphate buffer of pH 7.3 were used for each set of tests.

Phosphatide and Wax. These lipids were prepared from bacilli grown on synthetic medium according to the methods of Anderson, as described in a previous publication (24). The phosphatide was chilled and emulsified in distilled water by grinding in a mortar. The wax was emulsified by first dissolving it in chloroform and alcohol and adding to this an equal volume of salt solution, and subsequently evaporating the solvents over a water bath (25).

Carbohydrate. This was obtained from the filtrates of cultures on

synthetic medium by the method of Masucci, McAlpine, and Glenn (26).

The antigens were employed in amounts determined in each case by preliminary anti-complementary tests. Of the whole killed bacilli, .01 or .02 mg. was used as the test does for each tube. Of the defatted bacilli, 0.1 mg. was used. The culture medium filtrate was used in 1:5 dilution. The culture medium and bacillary protein were used in 0.025 mg. amounts. Phosphatide was used in .005 mg., and wax in 0.05 mg. quantities. The carbohydrate was used in varying dilutions from .01 to .002 mg. All antigen doses were contained in 0.1 ml. volumes.

Complement fixation tests. The technique of carrying out these tests has been described in a previous publication (24). Particular attention was paid to the anti-complementary properties of each of the antigens and in every case titrations for this activity were set up in the presence of diminishing quantities of complement along with each set of tests. Each serum was run in duplicate, one set without antigen, so that the anti-complementary activities of the individual sera could also be appraised. The tests were incubated at 4°C. for 18 hours. Serum dilutions were used in amounts of 0.1 ml. and the same quantity of each antigen was employed with all serum dilutions. The end point reading was made in the last tube showing 50% hemolysis.

RESULTS

Comparison of antigens. The efficacy of each of the antigens employed is indicated in Table 2 in which the results with tuberculous patients and with tuberculin-positive students are combined. The culture medium protein gave the greatest number of positive reactions of all the isolated derivatives of the bacillus tested. This has been found equally true in tests on experimentally infected animals (19). The similarly good result with culture medium filtrate was in all probability due to its high content of tuberculoprotein. The bacillary suspensions have been distinctly inferior to the protein solutions except in cases of active tuberculosis (See Table 4). Reactions with the phospholipid and wax have been too infrequent to be of any value. Tests with the carbohydrate have been uniformly negative.

The superiority of the protein antigens in these complement-fixation reactions was further indicated by the fact that in only one instance

was a positive reaction obtained with another type of antigen when the reactions with the protein antigens were negative. This exception was in the case of a tuberculin-positive student whose serum reacted to a dilution of 1:20 with the heat-killed bacilli but failed to react to defatted bacilli, culture medium filtrate, phosphatide or culture medium protein. In no instance did the bacillary protein give a positive reaction when the culture medium protein was negative among 14 cases in which the same serum was tested with both these antigens. In only one instance out of 35 tests did the culture medium filtrate give a positive reaction (in dilution of 1:20) when the culture medium protein was negative.

TABLE 2

Combined results of complement fixation tests in patients with active tuberculosis and in healthy tuberculin-positive subjects

TYPE OF ANTIGEN	NO. CASES TESTED	NO. CASES POSITIVE	PERCENT POSITIVE
Heat-killed bacilli	41	5	12.2
Defatted bacilli	41	8	19.5
Culture medium filtrate	41	14	34.2
Culture medium protein	50	22	44.0
Bacillary protein	22	3	13.6
Phosphatide	41	3	7.3
Wax	26	2	7.7
Carbohydrate	28	0	0

In view of these facts it was considered warranted to use the culture medium protein alone in canvassing the sera during the last half of the experiment.

Results in sarcoidosis. The complete data on the tests in cases of sarcoidosis are shown in Table 3. Those tests in which the sera were anticomplementary have been omitted. Six of the twenty-two cases gave positive reactions on one occasion. In only two of these cases was it possible to obtain a retest at a later date. In neither of them was a positive test obtained on the second or third sample of serum. In five of the six positive cases, positive reactions were obtained with more than one antigen. The superiority of the protein antigens is not as clearly demonstrated in this group as it was in the group of tuberculous subjects, probably because there were so few positive sera. In

TABLE 3

Complement-fixation reactions in 22 cases of sarcoidosis with antigens of M. tuberculosis

CASE NO.	DATE	HEAT-KILLED BACILLI	DEF-FATTED BACILLI	CULTURE MEDIUM FILTRATE	CULTURE MEDIUM PROTEIN	BACIL-LARY PROTEIN	PHOSPHA-TIDE	WAX	CARBOHY-DRATE
1	7-21-44	0	0	0	0	0	0	0	0
2	8-17-44	2	4	4		0	4		0
	12-13-46				0				
	9-16-47				0				
3	10-19-44	0	0	0	0	0	0	0	0
	4- 5-45	0	0	0	0		0	0	
4	10-24-44	0	0	0	0	0	0	0	0
5	12- 4-44	1	1	2			0		
6	12-18-44	0	0	0	0	0	0	0	0
	3-27-45	0	0	0			0	0	
7	3-10-45	0	0	0	0		0	0	
8	3-20-45	0	0	0			0	0	
9	5-28-45	0	0	0	4		0	0	
	8-11-45				0				
10	6-12-45	0	0	1	2		0	0	
11	6-12-45	0	0	2	2		8	0	
12	6-12-45	0	0	4	2		2	0	0
13	9-17-45				0				
	9-19-45				0				
14	1-31-46				0				
15	12-13-46				0				
	1-21-47				0				
16	2-25-47				0				
17	3- 3-47				0				
	7-17-47				0				

TABLE 3—*Continued*

ASE NO.	DATE	HEAT-KILLED BACILLI	DE-FATTED BACILLI	CULTURE MEDIUM FILTRATE	CULTURE MEDIUM PROTEIN	BACIL-LARY PROTEIN	PHOSPHA-TIDE	WAX	CARBOHY-DRATE
18	5-27-47				0				
19	6- 6-47				0				
	7-23-47				0				
	9-18-47				0				
20	6-20-47				0				
21	8-20-47				0				
	9-18-47				0				
22	12-18-47				0				

The figures indicate dilutions of serum used in 0.1 ml. amounts.

no instance did any other type of antigen give a positive result when the soluble protein antigens failed to do so.

Comparison of sarcoidosis with other groups. The cases of sarcoidosis have been compared in Table 4 with each of the other groups. For the sake of conciseness each individual antigen has not been listed separately. The antigens have been grouped into three types, viz., bacillary suspensions, protein solutions and lipid emulsions. This is justified by the close agreement between the results obtained with the sera of tuberculous patients with each antigen in the same group.

The last column of Table 4 summarizes the results in each group of cases with all the antigens used. It indicates a high proportion of positive reactions (61.7%) among cases with active tuberculosis. Healthy subjects with positive tuberculin tests gave a significantly smaller percentage of reactions (33.3%). There were no positive reactions among the control subjects who had negative tuberculin tests. The proportion of positive reactions in the cases of sarcoidosis (27.3%) was significantly lower than in the cases of active tuberculosis.

A comparison of the different groups of subjects in respect to their reactions with each type of antigen may also be made in Table 4. Only in the cases of active tuberculosis did the bacillary suspensions give an incidence of positive reactions comparable to those with the protein antigens. This was due to a high incidence of positive reactions

with the defatted bacillary antigen. Save for this one exception, the protein antigens gave more reactions than any other in all the groups.

Titers of antibody. The complement fixation tests have been carried out with empirically determined optimal quantities of each antigen. Arbitrarily assigning a unitary value to this quantity of antigen, the unit of antibody may be defined as the amount reacting with a unit of antigen. The units of antibody per milliliter of serum may be calcu-

TABLE 4

Comparison of complement-fixation in cases of sarcoidosis, tuberculosis, and healthy subjects

	TYPE OF ANTIGEN									ALL TYPES OF ANTIGEN	
	Bacillary* Suspensions			Protein† Solutions			Lipid‡ Emulsions				
	No. tested	No. positive	% positive	No. tested	No. positive	% positive	No. tested	No. positive	% positive	No. tested	% positive
22 cases of sarcoidosis	13	2	*15.4*	22	6	*27.3*	12	3	*25*	22	*27.3*
26 cases of active tuberculosis	11	7	*63.6*	26	16	*61.7*	11	4	*36.3*	26	*61.7*
30 Students with positive tuberculin tests	29	3	*10.3*	30	9	*29.7*	29	1	*6.7*	30	*33.3*
29 Students with negative tuberculin tests	27	0	*0*	29	0	*0*	27	0	*0*	29	*0*

* Includes the results with heat-killed bacilli and with defatted bacilli.

† Includes the results with culture medium filtrate, culture medium protein and bacillary protein.

‡ Includes the results with phospholipid and with wax.

lated from the highest dilution of serum giving a positive reaction. On this basis a comparison of the positive sera from each of the groups is presented in Table 5.

There is a significant preponderance of higher titers in the cases of active tuberculosis in comparison with the other groups. This is most clearly shown with the protein antigens where the numbers of positive results were highest. Sixty-seven per cent of the positive sera of tuberculous patients had titers of 40 units per ml. or higher when tested with the protein antigens. Only thirty-eight per cent of the positive

sera of patients with sarcoidosis had titers of 40 units per ml. with these antigens and none were higher. Twenty-three per cent of the positive sera of tuberculin positive students were 40 units per ml. A suggestive but less significant difference between the tuberculous and other sera is seen with the bacillary suspensions. There were too few positives with the lipid antigens to make a valid comparison.

TABLE 5

Distribution of titers of positive sera

	TYPE OF ANTIGEN												
	Bacillary Suspensions				Protein					Lipid			
Units of antibody per cc. of serum	10	20	40	80	10	20	40	80	160	10	20	40	80
6 Cases of Sarcoidosis	2	1	1		1	4	3				1	1	1
16 Cases of Tuberculosis	2	4	2	1	2	6	11	4	1	1	1	1	1
10 Students with Positive P. P. D.	2	2			7	3	3			1			

COMMENT

Our experience indicates that complement-fixation reactions with these antigens of *M. tuberculosis* are specific for tuberculous infection. No positive reactions were obtained in a group of healthy individuals who had negative tuberculin skin tests and had negative histories and x-ray examinations. The results were not complicated by concurrent syphilis in which false positive complement fixation with tubercle bacillus antigens has been reported (22). The considerable number of negative tests on patients with active tuberculosis indicates, however, that detectable levels of circulating antibody may not always develop or persist during and after the infection.

There is no established relationship between skin sensitivity to tuberculin and the presence of circulating antibodies to the tubercle bacillus (9). Each develops in response to tuberculous infection and each may wane with the passage of time. The skin sensitivity develops with much greater regularity and usually persists for a longer time in

infected human beings. In the experimental animal, however, the skin sensitivity often disappears late in the course of infection while circulating antibodies to bacillary protein persist (19). The disappearance of skin reactivity has been observed in moribund patients with far advanced tuberculosis and also as a transient occurrence during the course of certain acute infections (9). In this connection one of the cases of active tuberculosis in the present study is of particular interest. This was a young negro woman with extensive pulmonary disease and a negative sputum who had a negative tuberculin test to 10 mg. O.T. Her serum contained the highest titer of antibody (160 units per cc.) of all the cases in this series. Shortly after the test she developed signs of tuberculous meningitis and eventually died. Autopsy verified the diagnosis of pulmonary and meningeal tuberculosis. [This indicates that a high titer of antibody may be present in the serum of patients with active tuberculosis who have a negative tuberculin test just as in the experimental animals referred to above.]

There is good general agreement among observers that a substantial majority of patients with sarcoidosis have a negative tuberculin test (2, 6, 8, 20). The tests on the present series of cases are in agreement with general experience. Those who adhere to the theory of tuberculous etiology of sarcoidosis have referred to this fact as evidence of a "positive anergy", implying that the patient is desensitized by the infecting organism or its products (27). [Since there is no known effect of circulating antibodies on the state of tuberculin sensitivity, it might be anticipated that antibodies to the bacillus would still be present in the circulating blood of the sarcoid patient even in the absence of skin sensitivity, if the disease were indeed due to this agent.] Such a state of affairs has been observed in the tuberculous patient referred to above and in the experimental animal. The results of this study indicate that such an anticipation is not realized. The patients with sarcoidosis infrequently have circulating antibodies demonstrable by complement-fixation tests and those who do have antibodies tend to have a lower titer than patients with active tuberculosis.

The question remains as to the significance of antibodies to *M. tuberculosis* in the fraction of sarcoid patients in whom they were found. The group of patients studied was drawn at random from the hospital dispensary and offices of private physicians. They may not be

considered as representative of any limited social or economic group nor of the community as a whole. It would be fruitless to try accurately to predict the expected incidence of tuberculous infection among them. Since there is evidence that the incidence of positive tuberculin tests among cases of sarcoidosis is lower than it is among the population at large (2, 6, 8, 20), it is very likely that the incidence of past tuberculous infection among cases of sarcoidosis is higher than the incidence of tuberculin sensitivity, regardless of the etiology of this disease. The fact that about a quarter of the cases were sensitive to tuberculin suggests, therefore, that a somewhat larger proportion had had a past tuberculous infection. The presence of circulating antibodies to the tubercle bacillus in about a quarter of the cases is further evidence in favor of this deduction since only a third of the previously infected healthy subjects had antibodies. Altogether 8 of the 22 cases (36.4%) had *either* a positive skin test *or* positive complement-fixation. This suggests that a majority of them may have had a past tuberculous infection, a not unreasonable possibility in an urban group of this age distribution.

The etiological relationship of tuberculous infection to sarcoidosis, on the other hand, receives no support from this study. There is a large difference in the incidence of occurrence and in the titer of complement-fixation antibodies between the group of sarcoid patients studied and the patients with active tuberculosis. All the sarcoid patients had been biopsied relatively recently before the serological tests were done. In each case, the histological lesions were rich in epithelioid-cell tubercles. This is a feature which is a reliable indication of activity in tuberculous lesions. Moreover sarcoidosis is known to be a chronic, slowly progressive disease with evidence in many instances of continuous or recurrent spread over months or years. The clinical symptoms and observations in most of these cases indicated that the disease had recently progressed or was currently doing so. Therefore in order to show convincingly by serological means that the disease is of tuberculous etiology, it would be necessary to demonstrate a serological pattern that closely resembled that of active tuberculosis. This has not been done.

Unless patients with sarcoidosis have an impaired ability to produce or maintain circulating antibodies, the data presented here constitute

strong evidence against the tuberculous etiology of this disease. In view of the indications that patients with sarcoidosis suffer some disability in manifesting hypersensitivity to tuberculin following vaccination with avirulent bacilli (11, 12, 13, 28), it might reasonably be questioned whether they have the capacity to develop antibodies to the bacillus when infected as readily as other individuals (29). An attempt is being made at present to determine whether any difference exists in the ability of patients with sarcoidosis to develop antibodies to *M. tuberculosis* when vaccinated with avirulent bacilli in comparison with healthy individuals.

SUMMARY

Complement fixation reactions have been performed on the sera of twenty-two cases of sarcoidosis, using a variety of antigens prepared from the H37 strain of Mycobacterium tuberculosis. There was no clinical or pathological evidence of active tuberculosis among these patients and only four of them had positive tuberculin skin tests. Positive serological reactions were obtained on five (27.3%) of the cases. In contrast to this, sixteen (61.7%) of twenty-six cases of active tuberculosis gave positive serological reactions and the average titer was higher in this group than it was among the cases of sarcoidosis. Ten (33.3%) of thirty healthy control subjects, who had positive tuberculin tests, gave positive complement fixation reactions with a range of titers similar to that of the cases of sarcoidosis. It is believed, therefore, that the positive complement fixations observed among the cases of sarcoidosis may be due to a past tuberculous infection unrelated to the sarcoidosis. Unless patients with sarcoidosis have an impaired ability to develop complement fixing antibodies to the tubercle bacillus, the considerable difference in serological reactions between them and cases of active tuberculosis indicates a difference in the etiology of these diseases.

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CLINICAL THERAPEUTIC TRIAL OF A NEW DRUG[1]

The large number of new drugs which have been put forward in recent years as agents in the treatment of disease has raised many problems, none of which is more difficult of solution than the decision concerning their safety and efficacy in the treatment of the diseased patient. The conference today offers pharmacological and statistical advice on the design of therapeutic trials to the clinician who must ultimately be responsible for these decisions. The discussion will be opened by Dr. E. K. Marshall, Jr.

Dr. E. K. Marshall, Jr.: I should like to outline what data on the pharmacology of a new drug are, in my opinion, essential, and what are desirable before the drug is submitted to clinincal therapeutic trial. First, we have the question of the safety of the drug for the patient. A great deal, but by no means everything, about this safety factor can be learned from carefully planned experiments on the toxicity of the drug for animals. If the drug is found to exhibit definite signs of disturbance of the function of various organs, such as kidney, liver, hematopoietic or nervous system, it is extremely likely that sufficient dosage will cause the same type of toxic injury in man. On the other hand, where no toxic symptoms have been observed in animals except with very large doses of the drug, certain types of toxic symptoms may occur in man from small doses. These are mainly of the nature of allergic reactions, skin reactions and blood dyscrasias. These toxicity experiments should be done with a dosage schedule of drug similar to that which one expects to employ in the therapeutic use of the drug in man. A determination of the acute toxicity of the drug in animals (the toxicity resulting from a single dose) may give no information of value where one expects to give more than one dose of drug a day for several days in the clinical trials. Thus, quinine is much more toxic than quinacrine when a single dose is given, but the reverse is true when the drugs are administered several times a day over a period of several days. Also, it is important to determine toxicity on more than one species of animal. The therapeutic dose of quinine for man based on concentrations of drug in the plasma causes blindness in the dog

[1] Therapeutic Conference held April 30, 1949.

after a few days administration. In the monkey as in man this dose or multiples of it can be given without causing blindness or any serious toxic symptoms. An investigation of the toxicity of the 8–aminoquinolines (the only drugs known to be curative of vivax malaria) showed that in the case of these extremely toxic drugs data obtained in the monkey, but not those obtained in the dog, rabbit, rat or mouse, can be carried over to man. If possible, and particularly with extremely toxic drugs, both the monkey and dog should be included in the species used for toxicity studies. The doses of drug given to animals should include those large enough to cause symptoms, so that one may get some idea as to what to look for when the drug is given to man. Of great value in all these toxicity studies is the use of another drug of the same general chemical type, which has been given to man, as a standard of comparison.

While not essential, it is desirable to have some knowledge of the absorption, excretion, distribution, and fate of the drug before clinical trials are begun. This kind of information allows one to arrange dosage schedules in relation to the presence of drug in the organism and to study possible cumulation of the drug.

It is, of course, necessary to have a definite idea of the purpose for which the drug is to be given a clinical trial. The possible therapeutic value of the drug is obtained from animal experiments. These will be of many different types depending on the particular pharmacological actions of the drug. A potential chemotherapeutic agent is tested against some experimental infection in animals. If possible, this infection should be produced with the same species of parasite which causes human disease. If the drug under consideration is not a potential chemotherapeutic agent, the pharmacological studies may be of a variety of types in order to assess the possible therapeutic value of the drug. Since it is very difficult to conduct adequately controlled therapeutic studies on man, it is important to assess the therapeutic value of the drug in animals as carefully as possible as a basis for its therapeutic use. Here, again, a drug of similar nature already used in therapy should be used as a standard of reference for the new drug.

After animal experiments have been completed, the data obtained are checked by performing pharmacological studies on man. These are aimed mainly to confirm data on the absorption, excretion, dis-

tribution and degradation of the drug obtained in animals, and to assess in a preliminary way the toxicity of the drug for man. Since, many toxic reactions which are not seen in animals may occur in man, great care should be used in the initial administration of the drug to man. Somewhere around one-fifth or one-tenth of the dose which would appear from animal experiments to be safe in man might be given as a start. Only a single dose should be given initially, as unexpected untoward reactions are less likely with this procedure than with multiple doses. The drug is now ready for assessment as to its therapeutic value. The conduct of human therapeutic experiments with proper controls is by no means simple. The discussion of this phase of the subject, I shall leave to Dr. Merrell.

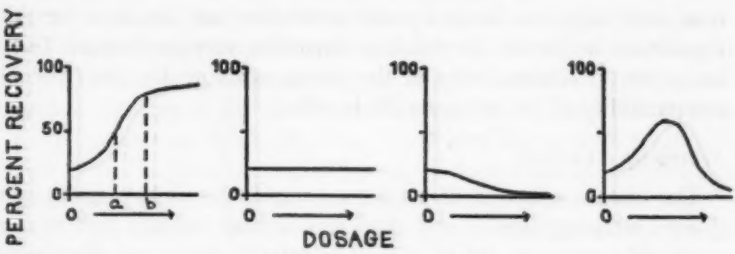

FIG. 1 FIG. 2 FIG. 3 FIG. 4

FIGS. 1–4

Dr. Margaret Merrell: The problem of human therapeutic tests on a drug is that of measuring some defined reaction or reactions, favorable or unfavorable, against varying doses of the drug. I shall discuss the issues first around the example of recovery from a specific disease.

We may visualize various patterns of dosage and percentage recovery. Some of these are illustrated in the following figures.

Fig. 1 shows the situation where about 20% recover without the treatment but the percentage increases to about 80% with increasing dosage. It never reaches 100% and beyond dose *b* there is no appreciable gain with increasing dosage. This is the sort of pattern we usually visualize with a drug that has definite therapeutic value.

It is well to keep in mind that quite different patterns may represent the actual situation. Fig. 2 is the case of a drug that does not affect

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the chances of recovery at all; fig. 3 shows the situation for the drug that affects survivorship unfavorably; and fig. 4 indicates a drug which has a therapeutic value up to a point, but beyond that it is unfavorable; that is, there is an optimum dose.

The first experiment in evaluating a new drug clinically may well be the test of the drug at some fixed dose against a zero dose. The hope is that we will be dealing with the situation in fig. 1 and it is desired to take a dose which is large enough (say dose *a*) to permit telling whether we are dealing with the case of fig. 1 or fig. 2 but not large enough to be dangerous should we actually be dealing with the cases of figs. 3 or 4. We would anticipate that if we get favorable results, then we would go on to a further examination of the dosage scale.

I shall confine my discussion to this first experiment (dose *a* against dose zero) since the issues I want to consider are the same for this experiment as for the further ones involving varying dosages. These issues are 1) comparability of the groups entering the test, and 2) comparability of the measures of the effect.

Setting up a Control

The first issue is that of getting a control, that is having the two groups receiving dose *a* and dose zero similar initially and in the course of treatment, for all things except the dose itself. Similarity refers to those things that affect the prognosis. However, it is impossible to identify all the elements that affect the outcome and therefore we require some process that will bring about similarity in our groups without attempting to categorize all the important factors and match the groups on those. We may do no matching at all, or we may specify certain characteristics for which we match the groups, such as sex, color, age etc., but within these classes we must have comparability for the numerous unspecified factors which may be influential.

This is achieved by some sort of randomizing process for assigning patients to the two groups, such as even and odd history numbers, or persons born in even and odd months of the year, or an assignment by lot. For this process to be effective, it is necessary to have a reasonably large number in each group, since we are counting on chance to achieve comparability in our groups. The question as to what is "reasonably large" depends upon the problem and the factors involved, but it is a rare problem indeed where a series of less than 20 per group is adequate

and often much larger numbers are needed. It is worth emphasizing that it is better to build up a well-controlled series in this way even if it has to be done gradually than to get a larger series poorly controlled. Large numbers in themselves are worse than useless if the groups are not comparable, since they encourage confidence in an erroneous opinion.

After the two groups have been set up it is well to compare them for various attributes to see that they are in fact similar for these factors. This constitutes a check on the procedure used. We must keep in mind that the object of the effort to achieve similar groups is that whatever difference we find in our results may be attributed to the drug and not to the fact that the groups were dissimilar in the first place.

It may be well to mention two or three types of "controls" that are not satisfactory. One is the historical control; for example the observation that in the pre-drug days, only m per cent reacted favorably and with the drug in use this is changed to n per cent. If everything remained constant in the situation, this would be all right, but there is no assurance that this is the case. There are differences in treatment aside from the specific drug, difference in type of patients presenting themselves, often due to the very fact that a drug is available; difference in the general economic and health set-up of the patients. A second unsatisfactory control is that made up of patients who refuse the treatment. One can think off hand of many ways in which such patients may differ from those accepting treatment. The impossibility of assessing the importance of these differences means that the results are incapable of interpretation and therefore the observations are wasted. Another control involving the same difficulties of interpretation is that obtained from another clinic not using the treatment.

In presenting the data on a clinical experiment the actual procedure used in selecting cases and assigning them to the two groups should be explicitly defined. If the study has been well-conducted, rules must have been laid down and these should be stated. It is difficult to evaluate the work reported in a paper when the description is given in such vague phrases as "consecutive cases." The absence of a more specific description leads naturally to a doubt as to the author's awareness of the difficulties surrounding the setting up of a good control and consequent doubt of the interpretation.

Once comparable groups have been established it is necessary that

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they should be treated in exactly the same way. It is of course for this reason that a placebo or some other drug is used to control various environmental and psychological factors that may influence the outcome. These influences may be very subtle but nevertheless potent and it is desirable whenever possible to have the person administering the treatment unaware of the identity of the drugs.

Measurement of Effects

The effects on which the drug is to be assessed should be defined at the outset and not left to be ferreted out when the results are in. Definitions and objective criteria should be laid down which will be alike for the two groups. If the effect is death or recovery this is easy, but even here certain criteria must be specified, such as the time cut-off of deaths to be counted, since the longer the patients are followed the more of them will die. The definition "death in hospital" is unsatisfactory because length of hospitalization may be a factor of consequence. It is important also to define any exceptions that may be made on cause of death, since exclusion of "non-specific" deaths may allow bias to creep in.

If the measurements are on x-ray findings, lesions, skin reactions, etc., it is important to make the criteria as objective as possible. However it is impossible in such measurements to rule out some subjective judgment on borderline cases. It is with this difficulty in mind that it is recommended that the person making the judgment have no knowledge of the treatment group to which the patient was allocated. The difficulty of avoiding unconscious bias is so great that the experimenter is in a peculiarly advantageous position if he can make his judgments unburdened by knowledge of the patient's treatment.

Illustrations

Numerous examples of the importance of these various features of a control could be cited from the medical literature. I want to mention certain features of three well-controlled clinical experiments by way of illustration. The first concerns the value of gelatin as an energizer. Uncertainty concerning its merit persisted for some years because of a number of inadequately controlled tests reported in the literature.

An interesting experiment was reported in 1942 (1) in which 33 volunteers from the football teams in an institution formed the test and control groups. The description of the experiment is in part as follows: "Two sets of tablets similar in appearance, shape, form, and taste were specially prepared . . . by a pharmaceutical house. The investigators knew only that one set . . . contained aninoacetic acid while the other set was a mixture of lactose and saccharose. The identity of the tablets was not revealed until the experiment had been completed, the final calculations made and conclusions drawn." The instrument used to measure work was a bicycle ergometer constructed so that work output could be translated into watt output per second. For 13 days there was a preliminary training and study period and then the tablets were given four times daily, with the explanation to the subjects that a test was being made to find out whether gelatin increased work capacity. The men were led to believe that all the tablets contained gelatin.

Figs. 5 and 6 show the results of the experiment. It will be seen that during the preliminary test period the work output was virtually level, but that both groups showed a marked increase while receiving the capsules. After 34 days the capsules were switched, so that the men who had been given the gelatin now received the placebo and vice versa. By this time the men had about fulfilled their potentialities and both groups levelled off. If the placebo had not been a part of this experiment, but instead, a "before and after" comparison had been made, it is almost inevitable that the conclusion would have been drawn that aminoacetic acid was an effective energizer. The authors emphasize the importance of the experimental design in the conclusion that "a study of the effects of a drug on fatigue must be meticulously controlled: the drug under study should be administered alternately with a placebo identical in physical characteristics, and the identity of each should be unknown to the subjects as well as to the investigators." From a wide variety of studies in other fields, it may be said that this statement holds generally and is by no means limited to studies on fatigue, where admittedly the psychological factor is an important one.

The second example is one of the war-time sea trials to assess the relative value of various motion sickness preventives. Two drugs and

a placebo were under test. For administrative reasons it would have been easier to use a single remedy on a given boat. It was argued that the boats were alike and were to run parallel courses. However, the persons designing the experiment insisted that all three remedies should be used on each boat and this was done. When the results were in, it was found that the men on one boat had lower rates for each of

Work Output Associated with the Administration of Aminoacetic Acid.

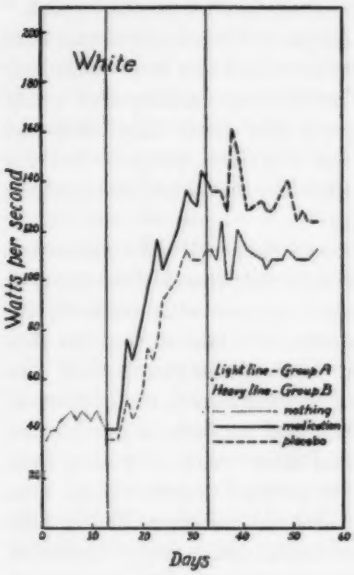

FIG. 5

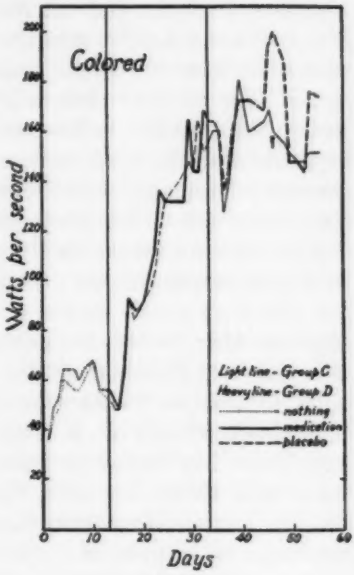

FIG. 6

the three remedies than the men on corresponding remedies on the other boat. Investigation revealed the fact that there was a difference in the ballast this boat was carrying. This was a fact which was quite inapparent to the experimenters and they would never have bothered to look for it if the boat had contained a single remedy. Had this been the case it would naturally have been inferred that this remedy was particularly efficacious, since the boats and courses run were alike.

Finally I want to cite a clinical experiment conducted by the

Medical Research Council on streptomycin treatment of pulmonary tuberculosis (2). The report merits study not only for the results but for the way the experiment was conducted. The first part of the report takes up a careful definition of the type of case accepted for study, how the cases were obtained, and how they were allotted to the streptomycin and control groups. "Determination of whether a patient would be treated by streptomycin and bed-rest (S case) or by bed-rest alone (C case) was made by reference to a statistical series based on random sampling numbers drawn up for each sex at each centre by Professor Bradford Hill; the details of the series were unknown to any of the investigators or to the coordinator and were contained in a set of sealed envelopes, each bearing on the outside only the name of the hospital and a number. After acceptance of a patient by the panel, and before admission to the streptomycin centre, the appropriate numbered envelope was opened at the central office; the card inside told if the patient was to be an S or C case, and this information was then given to the medical officer of the centre."

I have cited just one feature in the design of this study—that of assignment of patients to the two groups. The same scrupulous planning applied to other features of the study, and finally to the presentation of results. It is possible to find out from the report just what was done, and what the outcome was for patients falling into various diagnostic classes. The result is that a rather limited number of cases, only 107 all told, have served to give definitive results which one can interpret with confidence.

Statistical Aid

In dealing with clinical studies of this type, it is not at all uncommon for the clinician to seek statistical aid after the results are in, to find out whether differences are significant, or in some other way to have assistance in the analysis. If such a move is contemplated it is well to seek this assistance at the outset for there are no statistical maneuvers that will rescue a study that has not been well controlled. Statistical constants can be computed and it can be determined whether a difference is significant, but whether or not the difference is associated with the therapy or with the unknown or unthought of factor, such as ballast in the ship, is anybody's guess.

Practical Problems

Some of the recommendations and requirements listed above for a human experiment of this type may appear impossible of achievement. The question of the doctor's duty to the patient and the demands of the patient for a particular treatment immediately come to mind. Many of the potential difficulties do not arise, however, if the clinical experiment can be carried out very promptly following the necessary pharmacological tests. By "promptly" I mean as soon as the drug is ready to be tried on patients at all. The very first trials should be in terms of a well-controlled experiment.

It is easy to fall into the error of assuming that a new drug must be good and therefore it is unethical to withhold it from a patient. It must be remembered, however, that we may be dealing with the situation in fig. 3. It may well be that those from whom the drug is withheld are in fact the fortunate ones, and that by not setting up a control this fact is not immediately discovered.

Another reason for prompt action is that a good clinical experiment is far easier to achieve before the word of the new antibiotic reaches the news stands. And if it has, unfortunately, received early publicity, the drug may be relatively scarce at the outset, a fact that may be used to advantage.

If advance plans for the type of human experiment required are made while the pharmacologist is still carrying on the necessary laboratory work then, when this is complete, controlled trials can begin immediately and many of the hurdles which a delay is likely to erect, will not be there to obstruct the work.

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STUDIES ON THE INITIATION OF BLOOD COAGULATION

I. The Relationship of Platelets to the Clot-promoting Effect of Glass Surfaces[1]

ROBERT C. HARTMANN, C. LOCKARD CONLEY, AND JOHN S. LALLEY

(From the Clinical Microscopy Division, Department of Medicine, The Johns Hopkins University and Hospital, Baltimore, Maryland)

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Whole blood appears to contain all of the substances which are necessary for spontaneous coagulation. Blood obtained by techniques designed to prevent contamination with tissue juice or other extraneous material will clot spontaneously in a glass receptacle. In the classical theories of blood coagulation the initiation of clotting is attributed to the presence of thromboplastin, a substance which has the property of converting prothrombin to thrombin. An appreciable amount of effective thromboplastin is apparently not available in blood *in vivo* since intravascular clotting does not normally occur. The absence of effective thromboplastic activity from freshly drawn normal blood is further suggested by the great prolongation of the clotting time which is observed when blood is permitted to come in contact only with oiled or paraffined surfaces. Apparently on contact with certain foreign surfaces blood may undergo a change in which effective thromboplastic activity appears. The present studies are concerned with the nature of the alteration which takes place in blood on contact with glass surfaces.

According to the classical theory of blood coagulation the source of thromboplastin in blood is believed to be the platelets (1). While this belief has been widely accepted, some investigators have presented evidence suggesting that platelets are not necessary for the *initiation* of clotting (2, 3, 4, 5, 6, 7, 8). These workers believe that all of the components of blood required for spontaneous coagulation are present in platelet-free plasma. The use of silicone in blood clotting studies (9) has greatly facilitated the investigation of this problem. Nevertheless,

[1] This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

results obtained with this technique have been conflicting (8, 10, 11), and there is no agreement concerning the rôle of the platelets in the initiation of coagulation.

Previous studies have shown that when normal human plasma is rendered severely platelet-deficient, its clotting time in silicone-treated tubes is significantly longer than in glass tubes (8). Not infrequently essentially platelet-free plasma does not clot spontaneously in silicone-treated tubes although the same plasma clots readily in glass tubes. Contact of platelet-deficient plasma with a glass surface apparently results in an alteration which may not occur on contact with a silicone-treated surface. There are two possibilities concerning the manner in which glass surfaces serve to promote coagulation of essentially platelet-free plasma: (1) a few platelets remain and are essential for the initiation of clotting; or (2) the clotting process occurs independently of platelets. In the experiments to be described an attempt has been made to shed further light on this problem by observing the effects on coagulation of varying independently the concentration of platelets and the area of glass surface to which normal human plasma is exposed.

METHODS AND MATERIALS

Essentially platelet-free plasma was prepared by high-speed centrifugation of human blood at low temperatures, using silicone-treated[2] apparatus as previously described (8). No anticoagulant was employed. In most of these experiments the silicone-treated surfaces were reinforced by a film of silicone oil.[3] During the preparation of the plasma its temperature was not permitted to rise above 2°C. Special precautions were taken during the centrifugation period to ensure that the temperature of the plasma in the centrifuge cups did not exceed 2°C. Freezing of the plasma was not permitted to occur. Platelet-rich plasma was obtained in the same manner by low-speed centrifugation. Plasmas with different platelet concentrations were prepared by mixing platelet-deficient and platelet-rich plasmas from the same source. Platelet counts on platelet-rich plasma were performed using Rees-Ecker dilu-

[2] General Electric Dri-Film 9987.

[3] Silicone oil 9996-200 centistokes was kindly supplied by Dr. E. G. Rochow, Department of Chemistry, Harvard University.

ent, and on platelet-deficient plasma by direct microscopic observation of the undiluted plasma as previously described (8).

Clotting times of 1 ml. portions of plasma were determined at 37°C in scrupulously clean Pyrex test tubes (13 x 100 mm) using in most instances three tubes for each determination. The tubes were observed for a minimum of 24 hours.

Prothrombin times were determined by the one stage method of Quick using rabbit brain thromboplastin prepared according to the technique of Brambel (12). Recalcified plasma clotting times were measured at 37°C following the addition of 0.3 ml. of M/40 calcium chloride solution to a mixture of 0.3 ml. each of decalcified plasma and 0.85% sodium chloride solution. Decalcified plasmas were prepared by the addition of 10% by volume of M/10 sodium oxalate to native plasmas obtained by the silicone technique previously described. The prothrombin activity remaining in the serum after clotting was estimated by determination of the prothrombin time of the serum. 0.1 ml. of serum was added to a mixture of 0.1 ml. of rabbit-brain thromboplastin and 0.1 ml. of recalcified prothrombin-free plasma prepared by barium sulfate adsorption. The time required for clot formation at 37°C was then noted.[4]

Suspensions of macerated platelets were prepared from the sediment obtained by high-speed centrifugation of platelet-rich plasma. This sediment, which was composed of a solid, tenacious mass of platelets, was washed 6 times with 0.85% sodium chloride solution and then ground with a glass mortar and pestle. There appeared to be no loss of thromboplastic activity as a result of the repeated washings and maceration. From the platelet count of the original plasma and the volume of the saline in which the macerated platelets were finally suspended, the "platelet equivalent" of the suspension was estimated. Microscopic observation revealed that few if any intact platelets remained.

Crushed glass was prepared from Pyrex test tubes of the same lot used for clotting time determinations. Particles which passed through

[4] This procedure is not an accurate measure of residual prothrombin since the result may be influenced by other serum constituents. However, when thrombin is absent and changes in prothrombin are large, this technique seems to provide a satisfactory index of the amount of prothrombin disappearing during clotting.

a 32 mesh screen were treated with chromic acid, rinsed with copious quantities of distilled water, and dried in an oven.

RESULTS

The Clotting Time of Platelet-Deficient Plasma in Glass and Silicone-Treated Tubes

When human plasma was rendered severely platelet-deficient there was a marked difference between its clotting times in glass and in silicone-treated tubes. In Table I the data of 10 experiments are presented in which essentially platelet-free plasma showed no evidence of clotting in silicone-treated tubes at 37°C during a period of observation of at least 24 hours. In every instance, however, relatively prompt clotting occurred in glass tubes. The fact that these plasmas did not clot in silicone-treated tubes provides definitive evidence that no thrombin or effective thromboplastin was present in the plasma at the time of its introduction into glass tubes. It is apparent from the data in the table that there was no relationship between the clotting time in glass tubes and the number of platelets remaining.

TABLE I

Clotting Times of Severely Platelet-Deficient Plasmas in Glass and Silicone-Treated Tubes

EXPERIMENT	NATIVE PLATELET-DEFICIENT PLASMA		
	Platelets	Clotting time of 1 ml. portions at 37°-C	
		Glass tubes	Silicone tubes
	per cmm.	*min.*	*min.*
I	1	17	No clots
II	1	70	No clots
III	2	23	No clots
IV	3	49	No clots
V	3	54	No clots
VI	4	42	No clots
VII	6	97	No clots
VIII	10	39	No clots
IX	50	30	No clots
X	110	21	No clots

Plasmas were observed for clot formation for at least 24 hours.

TABLE II

Effect of the Addition of Crushed Glass on the Clotting Time of Plasma with Varying Platelet Concentrations

Experiment I

	PLATELETS (PER CMM)			
	8	167,500	385,000	670,000
Clotting time (min.)	4	4	4	3
	4.5	4	4	3.5
	5.5	4	4	4

Experiment II

	PLATELETS (PER CMM)			
	1	52,500	105,000	210,000
Clotting time (min.)	6	7	7	7
	7	8	7	8
	9	9	8	8

Experiment III

	PLATELETS (PER CMM)			
	23	16,000	156,000	312,000
Clotting time (min.)	4	4	4	4
	4	4	4	4
	4	4	4	4

Plasmas with different platelet concentrations were obtained by mixing platelet-deficient and platelet-rich plasma from the same source. 150 mgm of crushed glass was added to 1 ml. portions of plasma and the clotting time at 37°C determined in triplicate.

When no crushed glass was added the clotting times in glass tubes were much longer in each instance.

Effect on the Rate of Clotting of Varying the Platelet Concentration when the Glass Surface Area is Constant

When the glass surface area was relatively limited, as in ordinary test tubes, the clotting time of plasma appeared to be inversely related to the platelet concentration, if changes in the latter were large (8). However, when the surface in contact with plasma was increased by the addition of crushed glass, the rate of clotting became essentially

independent of the platelet concentration over an extremely wide range (Table II). Under these conditions the most platelet-deficient plasmas obtained clotted in approximately the same time as those containing several hundred thousand platelets per cmm.

TABLE III

Effect of Crushed Glass on the Rate of Clotting of Platelet-Rich and Platelet-Deficient Plasmas in Glass Tubes

Experiment I—Effect of different amounts of crushed glass on the rate of clotting of platelet-rich plasma (Platelets *410,000* per cmm)

	CRUSHED GLASS (MGM/ML. PLASMA)								
	0	10	25	50	100	150	300	500	800
Clotting time (min.)	9	6	5.5	4	4.5	3	3	2.5	2.5
	9	6	6.5	5	4.5	3.5	3	2.5	2.5

Experiment II.—Effect of different amounts of crushed glass on the rate of clotting of platelet-deficient plasma (Platelets *16* per cmm)

	CRUSHED GLASS (MGM/ML. PLASMA)								
	0	10	25	50	100	150	300	500	800
Clotting time (min.)	17	10	8	7.5	7	7	6.5	6	5
	40	11	9.5	9	9.5	7	7.5	6.5	6

Experiment III.—The relationship of particle size to the clot-promoting effect of crushed glass on platelet-deficient plasma (Platelets *6* per cmm)

	CRUSHED GLASS 150 MGM/ML. PLASMA				
	>16 Mesh	16 Mesh	32 Mesh	50 Mesh	100 Mesh
Clotting time (min.)	8	6	5.5	6	4
	9	7	6	3.5	3.5

The clotting times of 1 ml. portions of plasma were determined in duplicate at 37°C. In experiments I, II and III the subjects as well as the lots of crushed glass were different, so that the results are not strictly comparable.

Effect on the Rate of Clotting of Varying the Area of Glass Surface When the Platelet Concentration is Constant

The effect of adding varying amounts of crushed glass to platelet-rich and to platelet-deficient plasma is shown in Table III. With glass of the same particle size there was an inverse relationship between the amount of glass added and the clotting time, regardless of the platelet

concentration. Relatively small amounts of crushed glass were very effective in shortening the clotting time of severely platelet-deficient plasma. When a constant weight of crushed glass was added to plasma, the rate of clotting was inversely related to the glass particle size.

TABLE IV

Clot Accelerating Effects of Macerated Platelets on Platelet-Deficient Plasmas

Experiment I—Original Platelet concentration of plasma 3 per cmm

	TYPE OF TUBE						
	Glass	Silicone	Glass	Glass	Silicone	Glass	Silicone
	Crushed glass (mgm)						
	0	0	150	0	0	0	0
Macerated platelets (final concentration per cmm. of plasma)	0	0	0	200,000	200,000	2000	2000
Clotting time (min.) at 37°C	10	20	6	8	11	18	19
	17	49	6.5	8	12	18	27
	26	120	—	—	—	31	55

Experiment II.—Original platelet concentration of plasma 15 per cmm

	TYPE OF TUBE						
	Glass	Silicone	Glass	Glass	Silicone	Glass	Silicone
	Crushed glass (mgm)						
	0	0	150	0	0	0	0
Macerated platelets (final concentration per cmm. of plasma)	0	0	0	90,000	90,000	900	900
Clotting time (min.) at 37°C	12	15	6	7	—	7	19
	14	32	6	8	—	12	20
	19	51	6	8	—	16	51

0.1 ml. of suspensions of macerated platelets were added to 0.9 ml. of platelet-deficient plasma to give final concentrations of platelets recorded.

The clot accelerating effect of the macerated platelets was compared to that of crushed glass.

Clot Accelerating Effect of Macerated Platelets

The effect of macerated platelets on the clotting time of platelet-deficient plasma is shown in Table IV. Concentrated platelet suspension was less effective in accelerating coagulation than was crushed

glass. Furthermore, when crushed glass in adequate amounts was added to platelet-deficient plasma the clotting time was not further reduced by the simultaneous addition of macerated platelets. Diluted platelet suspensions had relatively little effect on the clotting time of platelet-deficient plasma in glass and silicone-treated tubes. In experiment I of Table IV, for example, the addition of sufficient platelet-material to provide a final concentration of 2000 macerated platelets per cmm., shortened the clotting time in silicone tubes only from 120 to 55 minutes. On the other hand, addition of 150 mgm. of crushed glass to this plasma containing only 3 platelets per cmm. shortened the clotting time to approximately 6 minutes. Concentrations of macerated platelets equivalent to many times the number of platelets originally present in centrifuged plasma failed to eliminate the difference between the clotting times in glass and silicone-treated tubes. In view of these results, it seems improbable that the increased rate of clotting of essentially platelet-free plasma in glass as compared to silicone-treated tubes can be attributed to the presence of a few remaining platelets.

Effect of Incubation on the Reactivity of Platelet-Deficient Plasma to Glass Surfaces

Platelet-deficient plasma which remained fluid in silicone-treated tubes at 37°C for 24 hours (Table I) occasionally clotted at the end of that time following the addition of crushed glass. However, in most cases such stored plasma was not clotted by glass. At times the reactivity of plasma to crushed glass was lost after only 6 hours incubation at 37°C. When plasma had been stored for a sufficient length of time so that it was no longer clotted by ground glass, it could still be clotted by rabbit-brain thromboplastin. Even when platelet-deficient plasma was stored at relatively low temperatures (2–7°C) for 24 hours, there was occasionally a loss of reactivity to glass surfaces at 37°C.

Relationship of Glass Surface Area and Platelet Concentration to the Amount of Prothrombin Converted During Clotting

When plasma clotted in silicone-treated tubes, the amount of prothrombin remaining after clotting was relatively large, regardless of the platelet concentration (Table V). In glass tubes on the other hand, there was an inverse relationship between the number of platelets

present and the quantity of prothrombin remaining. At a given platelet concentration the amount of prothrombin which disappeared during clotting was related to the glass surface area (Table VI). Even when

TABLE V

Relationship of Platelet Concentration to the Rate of Conversion of Prothrombin

		PLATELETS (PER CMM)						
		0	22,200	44,400	88,800	222,000	333,000	444,000
Residual prothrombin times (sec.) (4 hours after clotting)	Glass tubes	19	29	35	38	53	84	90
	Silicone tubes	18	21	23	23	27	26	24

The plasmas used in this experiment were derived from a single source, and allowed to clot at 37°C in glass and silicone-treated tubes. After 4 hours of incubation at 37°C the prothrombin times of the sera were determined as described in the section on methods

TABLE VI

Relationship of the Glass Surface Area to the Rate of Conversion of Prothrombin

TIME OF DETERMINATION IN RELATION TO CLOTTING	PROTHROMBIN TIME OF SERUM DERIVED FROM							
	Platelet-rich plasma* allowed to clot in the presence of crushed glass (mgm crushed glass/ml. of plasma)				Platelet-deficient plasma** allowed to clot in the presence of crushed glass (mgm crushed glass/ml. of plasma)			
	0	150	500	800	0	150	500	800
	Sec.	*Sec.*	*Sec.*	*Sec.*	*Sec.*	*Sec.*	*Sec.*	*Sec.*
Before	16	—	—	—	16	—	—	—
2 hours after	18	111	122	100	—	—	—	—
4 hours after	34	215	297	600	22	24	29	37

* Platelet-rich plasma contained 464,000 platelets per cmm.

** Platelet-deficient plasma contained 7 platelets per cmm.

Plasma was allowed to clot at 37°C in the presence of the amount of crushed glass indicated.

After 2 and 4 hours of incubation at 37°C the prothrombin time of the serum was determined as described in the section on methods.

plasma was severely platelet-deficient, this relationship persisted. Maximal conversion of prothrombin occurred only when both the platelet and surface factors were adequate.

Observations on the Mechanism of Action of Glass Surface in Coagulation

Crushed glass failed to cause the coagulation of oxalated plasma. The recalcified plasma clotting time was shortened after incubation

TABLE VII

Effect on the Recalcified Clotting Time of Oxalated Plasma of Incubation in Tubes of Different Surfaces and with Crushed Glass

	OXALATED PLATELET-DEFICIENT PLASMA (9 PLATELETS PER CMM.)						
	Incubated in glass tube		Incubated in tube with crushed glass, 450 mgm per ml.		Incubated in silicone-treated tube		
	Recalcified in Glass tube		Recalcified in Glass tube		Recalcified in Glass tube		Recalcified in Silicone-treated tube
Recalcified in presence of crushed glass (mgm)	0	150	0	150	0	150	0
Recalcified clotting time (min.)	12.5 13.5	7 7	8 8	7 7	13 16	7 7	16 17.5

Plasmas oxalated after the removal of the platelets were incubated at 37°C for 15 minutes with and without crushed glass. Following incubation the plasma was recalcified in glass and in silicone-treated tubes with and without added crushed glass.

TABLE VIII

Effect of Crushed Glass on the Recalcified Plasma Clotting Time in the Presence of Highly Diluted Rabbit-Brain Thromboplastin

Experiment I—Platelet-rich plasma (platelets *240,000* per cmm.)

	THROMBOPLASTIN CONCENTRATION							
	0		1:3200		1:800		1:200	
	Crushed glass (mgm/ml. plasma)							
	0	150	0	150	0	150	0	150
Recalcified clotting time (sec.)	205	96	130	88	72	58	46	39

Experiment II—Platelet-deficient plasma (platelets *35* per cmm.)

	THROMBOPLASTIN CONCENTRATION							
	0		1:3200		1:800		1:200	
	Crushed glass (mgm/ml. plasma)							
	0	150	0	150	0	150	0	150
Recalcified clotting time (sec.)	655	171	205	146	91	87	47	45

0.3 ml. of 0.85% sodium chloride solution or diluted thromboplastin was added to 0.3 ml. of oxalated plasma. The mixture was then recalcified with 0.3 ml. M/40 $CaCl_2$. Crushed glass was added just prior to recalcification. The platelet-rich and -deficient plasmas were obtained from the same source.

with crushed glass whether or not the glass was removed by high-speed centrifugation prior to recalcification (Table VII). The recalcified plasma clotting time of platelet-rich plasma was shorter than that of the same plasma rendered platelet-deficient (Table VIII). However, the clot promoting effect of crushed glass was as pronounced on platelet-deficient as on platelet-rich plasma. Increasing the surface area of glass was effective in shortening the recalcified plasma clotting time in the presence of diluted rabbit-brain thromboplastin. However, as the concentration of this thromboplastin was increased, the clot accelerating action of glass was diminished. With potent thromboplastin preparations such as are used in the Quick test, glass had no detectable effect. When oxalated plasma was clotted by thrombin,[5] glass had no demonstrable influence on the rate of the reaction.

DISCUSSION

A number of previous studies suggest that platelets are not essential for the *initiation* of coagulation (2, 3, 4, 5, 6, 7, 8). While some of the data presented are convincing, this conclusion has not been widely accepted. It is common belief that platelets are required for the initiation of clotting, but few investigators claim to have produced a spontaneously incoagulable platelet-free plasma from mammalian blood (10, 11, 13).

In the present paper experimental data show that essentially platelet-free plasma was often spontaneously incoagulable in silicone-treated tubes, although relatively prompt clotting invariably occurred in glass tubes. The absence of clot formation in silicone-treated tubes indicates that neither effective thromboplastin nor thrombin was produced during the preparation of platelet-deficient plasma. However, the occurrence of clotting of centrifuged plasma in glass tubes does not provide absolute proof that the effect of glass surface is not mediated through the platelets, since it is never possible to prove that platelets have been completely removed.

If the clot-promoting effect of glass surfaces is brought about by platelet disruption, it seems likely that a high concentration of macerated platelets would be very effective in accelerating coagulation. On the other hand increasing the area of the glass surface in contact with severely platelet-deficient plasma would be expected to have relatively

[5] Thrombin, Upjohn Company.

little clot accelerating effect. This, however, was not the case. Increasing the glass surface area was much more effective in reducing the clotting time of platelet-deficient plasma than was the addition of high concentrations of platelets which had been macerated on glass surfaces. These observations suggest that the presence of a few remaining platelets is not concerned with the clot-promoting effect of glass surfaces on centrifuged plasma.

While platelets appear not to be essential for the initiation of coagulation, they nevertheless play an important part in the clotting process. When plasma clots on contact with glass, the rate of conversion of prothrombin to thrombin is directly related to the platelet concentration. The clot-accelerating effect of platelets is not appreciable in plasma which is protected from contact with glass and similar surfaces. For rapid conversion of prothrombin both the surface factor and platelets are required.

Plasma which has been stored for relatively short periods of time may not clot on contact with glass. This observation suggests that the clot-promoting effect of glass is mediated by a labile component of plasma. Tests to determine the reactivity of plasma to glass surfaces evidently should be carried out while the plasma is fresh.

Platelet-free plasma is definitely hypo-coagulable as compared to normal plasma. In experiments to determine whether such plasma contains all of the substances necessary for spontaneous coagulation, optimal conditions for clotting must be provided. Those investigators who have reported that platelet-free plasma is spontaneously incoagulable have apparently not tested their plasmas under such optimal conditions. In the reported experiments (10, 11, 13), sub-optimal temperatures and minimal glass surface area have been employed. The experiments described in the present paper show that if coagulation of platelet-deficient plasma is sufficiently delayed, the plasma does in fact become spontaneously incoagulable, presumably because of deterioration of a plasma component.

Students of blood coagulation have for years been troubled by the problem of complete removal of platelets from plasma. A recent report (10) implies that many hours of centrifugation at high gravitational forces are required to remove all of the platelets from normal plasma.

However, in the present experiments approximately 99.995% of the original number of platelets in normal blood were regularly removed by centrifugation for only 10 minutes at about 17,500 g. Furthermore, on direct microscopic observation of the undiluted centrifuged plasma the few platelets remaining were observed to settle to the surface of the counting chamber. No evidence was found to support the belief that these platelets were remarkably less dense than the average platelets. It seems likely that these platelets were derived from the centrifuged sediment as a result of the slight agitation inevitably produced by withdrawal of the supernatant plasma.

The manner in which glass surfaces promote coagulation of platelet-free plasma remains to be clarified. It appears that on contact with glass, plasma undergoes a change in which effective thromboplastin is made available. Studies on patients with certain disorders of blood coagulation (8) make it seem likely that a specific protein is present in normal blood as an inactive thromboplastin and is converted to an active form on contact with a glass surface. No evidence was found that glass surfaces affect the final stage of coagulation.

SUMMARY

Normal human plasma of varying platelet concentration was prepared without anticoagulant by centrifugation of blood in silicone-treated apparatus. Essentially platelet-free plasma clotted relatively promptly after transfer to glass tubes, but in silicone-treated tubes coagulation frequently did not occur.

Increasing the glass surface area in contact with plasma shortened the clotting time markedly regardless of the platelet concentration. Crushed glass was more effective in shortening the clotting time of platelet-deficient plasma than was a suspension of macerated platelets.

The clot promoting effect of crushed glass on centrifuged plasma seems better explained by an alteration produced in the platelet-free plasma itself rather than by disruption of a few remaining platelets. After storage for relatively short periods of time, plasmas often failed to clot on addition of crushed glass.

In plasma clotting in contact with glass, the rate of conversion of prothrombin was directly related to the platelet concentration.

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BOOK REVIEWS

Conditional Reflexes and Neuron Organization. By JERZY KORNORSKI. *Cambridge University Press*, 1948, pp. 267 + XIV, 18 s.

The reaction to Pavlov's investigations by the conditional reflex method on the "higher nervous activity" has been a curious and interesting one. For a long time little attention was paid to his discovery at the turn of the century of the conditional reflex, partly on account of the language difficulty, partly because of the lack of scientific intercourse between Russia and the West. When Pavlov's concepts reached this country they were adopted by some psychologists as the nucleus of a new school of thought—Behaviorism, the chief exponent of which was John B. Watson. The overzealousness of its proponents in denying the existence of everything not measurable has caused this school to fall into disrepute. At the present time the opinion in this country of Pavlov's work ranges from an active hostility to its being hailed as one of the greatest discoveries in the history of science. There are many reasons why this work has not been actively taken up in this country since Watson carried out some experimentation by this method on the human being at the Johns Hopkins Hospital. One reason is the lack of trained investigators; another is the general reluctance of physiologists to undertake the chronic experiment requiring a study period of months or even years with all the difficulties of maintaining healthy animals over their life span. In spite of the general acceptance of the importance of the conditional reflex, the lack of active investigators in the field, as well as the novelty of the concepts has resulted in the failure of Medicine to incorporate the data and the concepts into physiology, neurology, psychiatry or even psychology.

Kornorski has written a masterful criticism from a constructive point of view of the Pavlovian concepts. In fact this is one of the few worthwhile evaluations that has been given of the Pavlovian work. Kornorski's purpose is to integrate Pavlov's data with that of Sherrington "in the hope that this work will do something to bridge the gulf between their respective achievements" (from his dedication of the book). His treatise is practically essential for any serious student of Pavlovian Methodology. He goes into detailed discussions of many of the basic Pavlovian concepts, showing their relationships to Sherrington's work on spinal reflexes. With rare insight and with the competence of a first-class investigator in the field he has given us not only a scholarly but probably the most important evaluation we have of the Pavlovian work. Kornorski makes many suggestions as to how the Pavlovian concepts should be re-cast to fit better with Sherrington's material. Especial credit is due Kornorski for having composed this book in the wake of the destruction of his native land (Poland) as well as of his own laboratory.

W. H. G.

Clinical Orthoptics By Mary Everist Kramer, Ernest A. W. Sheppard and Louisa Wells-Kramer 475 pp. $8.00. *The C. V. Mosby Co.*, St. Louis, 1949.

This book has been written as a textbook for orthoptic students and as a reference book for graduate orthoptic technicians. It fulfills both of these functions well. The entire problem of orthoptics has been considered both from the clinical standpoint and also from the point of view of the basic sciences. There has been a careful selection of material so that all of the phases of orthoptic work are discussed.

The style of writing is clear and concise, and the book is easy to read. The questions at the end of each chapter should prove a great help to the orthoptic student in systematically reviewing the topics covered. As a whole the illustrations are excellent, but some of the sketches in Chapters 1 and 2 are unnecessarily diagrammatic. Each chapter of the book contains an excellent list of references where the student can obtain more detailed information.

There has been a great need for such a book on orthoptics written expressly for the orthoptic technician.

W. C. O.

Campbell's Operative Orthopedics By J. S. Speed and Hugh Smith, Second edition, 1643 pp. $30.00 2 Volumes, *C. V. Mosby Co.*, Publishers, 1949.

The second edition of Campbell's Operative Orthopedics, like the first edition, is an excellent compendium of operative procedures used in orthopedic surgery. For the student, interne and resident, the value of such a compendium is obvious. For the specialist, it is important that a good publication of this type be available. In a field in which there appear in rapid succession new operations and new techniques, it becomes necessary to have a satisfactory reference book. The present edition constitutes an up-to-date presentation which is particularly valuable because the procedures enumerated are credited to those who described them and the original articles are listed for reference.

There are many improvements in the new edition. The material presented is brought abreast of the current time, with the result that there is much more printed matter. Since this is bound in two volumes, the handling of a single large cumbersome book is avoided.

Many new and excellent illustrations have been added, and on some special subjects, new chapters have been included. Several sections have been written by men who are particularly well qualified and some of these sections are very well done. But the greatest change is probably in the literary form. Instead of the compact, almost laconic, style used in the earlier edition, there is a fluency that makes the text more readable. Furthermore, the authors make the presentation a more personal one by discussing their own experiences with some of the procedures described and the reader profits by their carefully considered, as well as extensive, observations.

I. W. N.

Fundamentals of Internal Medicine—3rd Edition By WALLACE M. Yater. 1451 pp. $12.00 Appleton-Century Crofts, Inc., New York.

What is the purpose of a one volume textbook of medicine? In Dr. Yater's view, it should "make readily available in simple form . . . the essentials of . . . internal medicine" in accordance with the concept of "simplification and conciseness with elimination of theoretical and redundant material." To this end, Dr. Yater and his associates have written a well-integrated, readable book, emphasizing clinical descriptions and the essentials of therapy. Many clear tables and illustrations are included, and the physical format of this volume is pleasing to the eye. Little space is spent on the mechanisms of the production of symptoms, nor on the problems of etiology and pathogenesis. These are frequently dismissed with a few short sentences which fail to introduce the student to the many unsolved problems in internal medicine. Moreover, these didactic synopses are frequently misleading. For example, hemophilia is said to be associated with "an increased resistance of blood platelets to disintegration", a view far from universally shared. Nor is the life span of the normal red blood cell thought to be 2 to 6 weeks by most observers. This text is an excellent précis of symptoms and signs, but will not help the student to think in terms of the physiologic mechanisms involved.

O. D. R.

Medicine of the Year 1949. Editorial Direction, JOHN B. YOUMANS, M.D., Dean, College of Medicine, University of Illinois. 143 pp. $5.00. *J. B. Lippincott*, Phila., Pa.

An immediate reaction to a book which attempts to give the "spot news of medicine", which attempts to furnish the "latest bulletins that indicate the direction in which any phase of medicine is going at the moment", is for many reasons not particularly favorable. Perhaps it is that one recognizes the inherent difficulty of evaluating advances within the year in which they arrive. Yet, after reading through "Medicine of the Year", one is forced to the conclusion that the authors and particularly the editor, have done an excellent job. So diverse is the material that it is, of course, not possible for one person to adequately evaluate the different contributions to internal medicine, obstetrics, pediatrics and surgery with all of its specialized branches. However, within the limits of this reviewer's knowledge, whenever data presented is debatable the authors have so indicated, whenever questions are unsettled there has been no attempt to force them into a mold of decision. At the same time, recent clear-cut advances are presented concisely and factually. The recent evaluation of antibiotics is carefully covered throughout, without any mistakes being apparent even some time after the original articles were apparently written.

There are a few scattered misprints and misspellings, but these are minimal. It will, perhaps, be more difficult to write the issue next year when there will be less reason to go back a few years in evaluating recent progress, but certainly this first try was worthwhile and the next issue will be looked forward to with interest.

F. B. B.

Nutrition and Diet in Health and Disease. BY JAMES S. MCLESTER 5th edition, 800 pp. $9.00. *W. B. Saunders, Phila., Pa.*

Nutrition and Diet in Health and Disease comprehensively reviews the present state of knowledge of this subject. Dr. McLester discusses the physiology of digestion, the metabolism of food, the composition of common foodstuffs, and the dietetic requirements of normal adults and infants. The author then describes the diets suitable for the treatment of disease. In each case, a brief summary of knowledge concerning alterations in metabolism is followed by a general discussion outlining suitable dietetic regimens, and the rationale for their use. In many instances, sample menus are provided as well. The text is supplemented by many tables, the most useful of which give the composition of foodstuffs.

This is the fifth edition of this excellent and most readable book, and most of the text has been brought up to date. As is almost inevitable, the revision has been more thorough in some sections than in others. The use of rice and low salt diets for the treatment of hypertension is discussed and evaluated with commendable caution. However, no mention is made of the use of detergents to facilitate intestinal absorption. In general, the dietetic recommendations are sound. In a few cases, however, one may take issue with the author's point of view. For example, a diet rich in calcium is not ordinarily believed to be of value in hemophilia, a disorder in which calcium metabolism is not abnormal.

Each chapter is followed by an excellent bibliography, and each section by a list of pertinent reviews covering the subject discussed. This book can be highly recommended as a text useful to students, practitioners and dietitians. It is unfortunate that many misprints are present, and the type of many of the tables and menus is badly blurred.

O. D. R.

Observations on the Pathology of Hydrocephalus (Medical Research Council—Special Report Series No. 265) BY DOROTHY S. RUSSELL. His Majesty's Stationery Office, London: 1949, Price—Six shillings net, 138 pages.

This masterly monograph from the hand of Dr. Dorothy Russell will need no introduction to those who know her classical article of fourteen years ago. At that time she demonstrated the keen observation and sound reasoning which characterize the present comprehensive report on hydrocephalus. She draws on a wealth of morbid anatomical material in exemplifying the categories of her classification. The latter is based on the pathological lesions responsible for the obstruction in the cerebrospinal pathway. Indeed, the most important conclusion she draws from her detailed studies is that in every case of hydrocephalus some such form of obstruction can be demonstrated if careful search is made.

Amongst the types of maldevelopment productive of hydrocephalus, the author stresses the forking atresias and webs of the aqueduct, and the membranes about the fourth ventricle. She reiterates her evidence against the traction hypotheses in the causation of the Arnold-Chiari malformation. Her examples of the mechanisms of obstruction associated with neoplasms are well chosen.

It is in the consideration of the more obscure forms of hydrocephalus, however, that the thoroughness of her survey seems most impressive. It lends great strength to the view that the vast majority of cases rest on an inflammatory basis. This may follow the extravasation of blood or be an insidiously-developing sequel to actual infection. The common sites of such lesions, often found only by careful search, are described. The implications of this discussion of inflammatory causation will be clear to everyone dealing with the child or adult patient. A minor query in regard to the validity of the author's distinction between developmental and acquired aqueductal atresia does not detract from this.

In summary, this monograph is strongly recommended to all, and its author warmly congratulated. The quality of the illustrations is in keeping with the subject material.

J. W. M.

Studies on Hookworm Disease in Szechwan Province, West China. By K. Chang and co-workers. American Journal of Hygiene Monographic Series no. 19, May 1949. *Johns Hopkins Press.* pp. 152, $3.00.

There is a prevalent tendency on the part of the educated physician and layman to believe that the battles against some of the common scourges of mankind have been won. The campaigns against hookworm in this country, successful as they were, should not blind us to the fact that in great areas of the world this simple preventable disease remains one of the most important. A clear example of this situation is presented by Dr. Chang and his collaborators who report on the prevalence of hookworm in West China—in the province surrounding the former capital, Chungking. Given a sandy soil, a moderate temperature without extremes, a sufficient moisture and habits of a people which allow for a constant contact between human feces and the bare skin of man—and hookworm becomes a major problem. Such is the case in the corn-sweet potato regions of Szechwan, where the incidence of positive stools approaches 100%. Much more important, as was realized years ago by the Rockefeller Foundation workers, is the parasite load of the community. It is more important to know if a number of people are carrying heavy loads of parasites, than it is to know how many of them are infected. In certain areas of this province, well over 50% of the population carried more than 3000 hookworm eggs per cc. stool. The exact importance of this is of course difficult to assess, for the combination of heavy infection plus a poor diet is the usual combination. However, roughly, 10% had geophagia, and the hookworm load was directly correlated with the percent of hemoglobin, which was often very low.

The variable effects of slight differences in horticultural practices is brought out by a difference in the problem of hookworm infection in Eastern China, where some twenty years ago Dr. Cort and collaborators demonstrated that the cultivation of mulberry trees was associated with a high incidence of hookworm. In contrast in Szechwan, the disease is not particularly serious in mulberry areas. This is because, in the latter, mulberry trees are scattered in different fields, are not inundated with night soil in the same way, and because the trees are allowed to

grow. This latter means that the leaves have to be picked by climbing a ladder. In Eastern China, the opposite of all of these conditions leads to a severe hookworm problem.

To make the report living the authors have given a number of case histories. "Li, by name, male, a farmer, aged 26, came to our field clinic on August 19th, 1941, for the treatment of *Lan Hwang Ping* (lazy yellow disease). The patient was very weak, extremely anemic, exceedingly pale, expressionless; the voice was low; he breathed with difficulty but was mentally clear. He was exceedingly edematous all over the body, there was extreme edema of the lower extremities, his legs were very puffy and heavy and he could not walk with ease. The scrotum and tip of the penis were also extremely edematous. Fecal examination showed the following: Protozoa, negative; Ascaris, negative; Trichuris, 300 eggs per cc.; hookworm, 45,400 eggs per cc. of feces (three counts were made and averaged). A total of 637 worms, of which 602 were Ancylostoma duodenale and 35 were Necator americanus, were collected from the patient. His hemoglobin was only 10%".

This is a good, well-illustrated and worthwile monograph which should be at least perused by all who are actively interested in the health of the world.

F. B. B.

The New York Academy of Medicine: Its First Hundred Years. PHILIP VAN INGEN, M.D. 573 pp. $10.00 *Columbia University Press*, Morningside Heights, New York.

This is an interesting and amusing chronicle of the meetings and doings of the "fellows" of the New York Academy of Medicine. It does not pretend to be history in the broad sense, and one may search in vain for information about some of the famous medical men of New York beyond that which has a direct bearing upon their activities within the Academy.

Yet along with the amusing anecdotes and disagreements as to who should be censured for "seeking the advice and consulting with a homeopath", there is real information concerning the opinion of a body of the leading medical men at a particular time. The Academy was founded at the turn of the century. It was also at this time that Shattuck wrote his famous report on sanitary conditions in Boston and John Snow in England was just beginning to write about the contagiousness of cholera. Thus it is of interest to read about the discussions on cholera in the year 1849. "The year might well be called the cholera year" for little else was discussed in the months of February, March, and April.

Dr. Stewart stated that in connection with the origin and mode of propagation of the disease and particularly with that form of it which prevailed at the Quarantine at Staten Island, at the City of New Orleans, and in the "Western Country," circumstances had occurred which were not yet fully and satisfactorily explained. He therefore moved, and it was resolved, "that it is premature and inexpedient

for this Academy to pronounce at the present time any positive opinion in regard to the contagious or non-contagious nature of cholera."

However, in 1866 Dr. Harris, an experienced health authority, reported on "Sanitary Police Measures 2." He insisted that cholera was spread through the diarrhoeal discharges of patients, that disinfection was a simple matter, and rigid regulations should be enforced to disinfect permanently all ejecta, and to provide strictest cleanliness of person and premises, including privies, water closets and cess pools. If this were done there should be no fear or hesitancy in caring for the sick. At the same time Dr. Squibb reported on disinfectants, the best being heat and chlorine for the effluvia, together with lime, and a mixture of lime and charcoal. For disinfection of cellars smoke from green wood was best.

The Academy was founded in 1846 with several objects in view. "First, the separation of the regular from irregular practicioners. Second, the association of the profession proper. Third, the promotion of the character, interests and honor of the fraternity, etc., and fourth, the cultivation and advancement of the science, by our united exertions for mutual improvement and our contributions to medical literature."

Since this book is more a chronicle of the meetings, it is not always easy from this record alone to see to what extent the Academy has attained each one of those goals. Admittedly the importance of the different points has changed. At any rate, the book makes for enjoyable reading.

F. B. B.

BOOKS RECEIVED FOR REVIEW

Advances in Surgery. By WILLIAM DEW. ANDRUS, Chairman, Editorial Board. 554 pp. $11.00 *Interscience Publishers, Inc. 215 Fourth Ave. New York 3.*

Clinical Cystoscopy. By LOWRAIN E. MCCREA in two volumes, 2nd edition. 1152 pp. $28.00 *F. A. Davis Co., Philadelphia 3, Pa.*

Diagnostic Tests for Infants and Children. By H. BEHRENDT. 529 pp. $7.50. *Interscience Publishers, 215 Fourth Ave. New York 3.*

Medical Etymology. By O. H. PERRY PEPPER. 263 pp. $5.50 *W. B. Saunders Co.*

Oral and Dental Diagnosis. New 3rd edition. By KURT H. THOMA. 563 pp. $9.50. *W. B. Saunders Co. W. Washington Square, Phila. 5, Pa.*

Psychosomatic Medicine. 2nd edition. By EDWARD WEISS AND O. SPURGEON ENGLISH. 803 pp. $9.50. *W. B. Saunders Co. W. Washington Square, Phila. 5, Pa.*

The Premature Infant: Medical and Nursing Care. By JULIUS H. HESS, M.D. AND EVELYN C. LUNDEEN, R. N. 381 pp. $6.00. *J. B. Lippincott Co., E. Washington Square, Phila. 5, Pa.*

Geriatric Medicine. 2nd edition. By EDWARD J. STIEGLITZ. 773 pp. $12.00 *W. B. Saunders Co. W. Washington Sq., Phila. 5, Pa.*

Bensley's Practical Anatomy of the Rabbit. 8th edition. By E. HORNE CRAIGIE, PH.D. 391 pp. $4.00. *The Blakiston Co., Phila. 5, Pa.*

Female Sex Endocrinology. By CHARLES H. BIRNBERG. 134 pp. $4.00. *J. B. Lippincott Co. E. Washington Sq., Phila. Pa.*

Medicine of the Year. 1949 Editorial Direction of JOHN B. YOUMANS, Dean, College of Medicine, University of Illinois. 143 pp. $5.00. *J. B. Lippincott Co. E. Washington Sq., Phila. Pa.*